Cassava Common Mosaic Virus Infections of Chaya (*Cnidoscolus aconitifolius*) in Yucatán, Mexico

M. S. ELLIOTT, Biological Scientist III, and F. W. ZETTLER, Professor, Department of Plant Pathology, University of Florida, Gainesville 32611

ABSTRACT

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Cassava common mosaic virus (CCMV) was detected in 23 of 33 samples of chaya (*Cnidoscolus aconitifolius*) collected at 14 of 17 locations in Yucatán, Mexico, in August 1985. The CCMV isolates were serologically indistinguishable from a strain of this virus described previously from chaya in Florida.

Additional key words: DAS-ELISA, ELISA, *Manihot esculenta*, *Nicotiana benthamiana*, SDS immunodiffusion, serology

Chaya (*Cnidoscolus aconitifolius* (Miller) I. M. Johnston subsp. *aconitifolius* cv. Chayamansa) is an edible member of the Euphorbiaceae indigenous to Mexico. It has been cultivated since pre-Columbian times and today is typically found in groups of two to five plants in home gardens throughout the state of Yucatán, where it is cultivated as a leafy vegetable (2,3). Chaya plants growing in Florida were shown to be infected with a strain of cassava common mosaic virus (CCMV-Ch) that infects cassava (*Manihot esculenta* Crantz) but is serologically distinct from CCMV isolates described elsewhere (6). The objectives of this study were to determine whether the incidence of CCMV in chaya is high in Yucatán and whether chaya viral isolates collected there are antigenically similar to the CCMV-Ch isolate previously described from Florida.

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MATERIALS AND METHODS

Surveys. Surveys were made on 20–22 August 1985 within a 70-km radius of Homún, Yucatán (Fig. 1). Leaves were collected, regardless of symptoms, from 33 cultivated chaya plants and from six noncultivated plants of *C. aconitifolius* subsp. *polyanthus* (Pax & K. Hoffm.). Also, 23 samples of cassava were collected from the Uxmal Agricultural Experiment Station (Centro de Investigaciones Agrícolas de la Península de Yucatán). These cassava plants were part of a germ plasm collection and represent cultivars obtained outside Mexico. Two additional cassava and single wild specimens of *M. esculenta* and *M.*

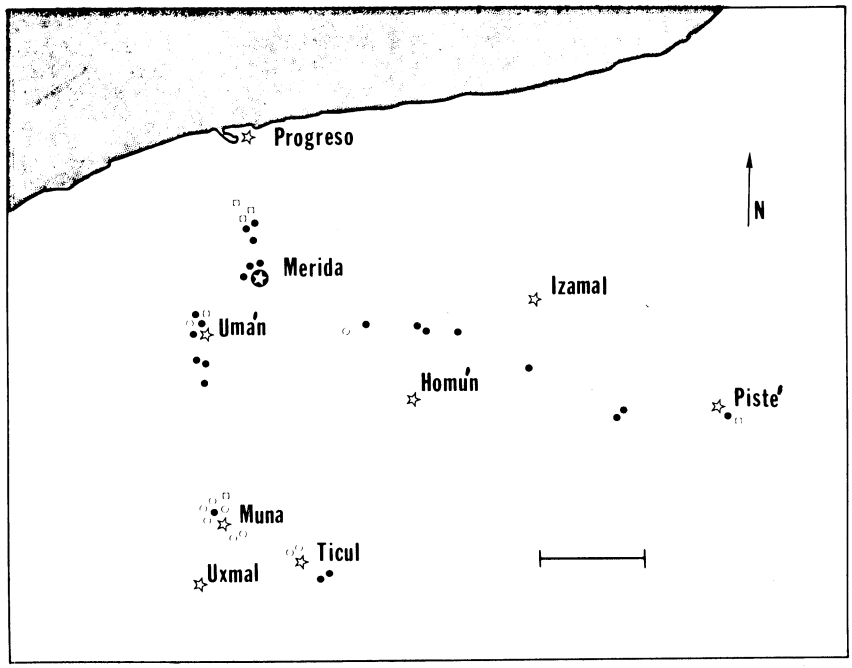

Fig. 1. Locations surveyed for cassava common mosaic virus in Yucatán, Mexico. Stars represent towns; black circles, infected chaya plants; open circles, noninfected chaya plants; and open squares, noninfected wild *Cnidoscolus aconitifolius* subsp. *polyanthus* plants. Scale bar = 25 km.

Table 1. Incidence of cassava common mosaic virus in chaya (*Cnidoscolus aconitifolius*) plants sampled from 17 locations in Yucatán, Mexico

Sample no.	Location	Serology		
		SDS[a]	ELISA[b]	EM[c]
1	Dzan	+	1.053*	···
2	Dzan	+	0.393*	···
3	Dzibichaltún	+	1.868*	+
4	Dzibichaltún	+	0.566*	···
5	Hoctun	+	1.650*	···
6	Kantunil	+	>2.000	+
7	Libre Unión	+	>2.000	+
8	Libre Unión	+	1.280	+
9	Mérida	+	0.818*	+
10	Mérida	+	1.805*	···
11	Mérida	+	0.320	+
12	Muna	−	0.011	···
13	Muna	−	0.020	···
14	Muna	−	0.026	···
15	Muna	−	0.000	···
16	Muna	+	1.497*	···
17	Piste	+	0.450	+
18	San Bernadino	+	1.589*	···
19	San Jose Tib-Ceh	−	0.066	···
20	San Jose Tib-Ceh	−	0.012	···
21	Tahmek	+	0.475	+
22	Tahmek	+	1.610	+
23	Ticopo	−	0.000	···
24	Ticul	−	0.000	···
25	Ticul	−	0.000	···
26	Umán	−	0.053*	···
27	Umán	+	0.730	+
28	Umán	+	0.114*	···
29	Umán	+	0.234*	+
30	Xcam	+	1.865*	···
31	Xtepen	+	0.310	+
32	Xtepen	+	0.384*	···
33	Yaxcopoil	+	0.310	+
34	Healthy wild *Cnidoscolus*[d]	−	0.002*	···
35	Healthy cultivated chaya[d]	−	0.034*	···
36	CCMV-Ch infected chaya[d]	+	0.207	···

[a] SDS immunodiffusion tests: + = positive reactions against CCMV-Ch antiserum; − = no reaction.
[b] Double antibody sandwich ELISA A_{405nm} values. Values marked by asterisks represent averages of five or more wells; values without asterisks represent two or three wells.
[c] Flexuous rod-shaped virus particles seen in negatively stained leaf extracts.
[d] Controls; the wild *Cnidoscolus* was *C. aconitifolius* subsp. *polyanthus*.

carthaginensis (Jacq.) Muell.-Arg. were collected from near Mérida. The specimens of wild *Cnidoscolus* spp. and *Manihot* spp. were identified by R. Orellana (Centro para Investigación Científica del Yucatán, Mérida).

Serology. The CCMV-Ch antiserum described by Zettler and Elliott (6) was used to test all chaya and cassava samples collected. The antiserum used in immunodiffusion tests was prepared against degraded virus and gave homologous antigen titers in agar-gel immunodiffusion tests of 1/8 and 1/16 with chaya and cassava leaf extracts, respectively (6). All immunodiffusion tests with CCMV-Ch antiserum were repeated at least once. Some of the samples were also compared in immunodiffusion tests with antiserum to a cassava CCMV isolate (CCMV-BPL) provided by B. L. Nolt (CIAT, Cali, Colombia). The CCMV-BPL antiserum gave a homologous viral antigen titer from infected cassava of 1/32. Reference antigens included 1) chaya leaf extracts infected with CCMV-Ch, 2) cassava leaf extracts infected with CCMV isolates provided by E. W. Kitajima (Dept. Biol. Celular, Univ. Brasilia, Brasilia, Brazil) and A. S. Costa (Inst. Agron., Campinas, São Paulo, Brazil), and 3) noninfected leaf extracts of cassava, *Nicotiana benthamiana* Domin, chaya, and *C. aconitifolius* subsp. *polyanthus*.

Leaf tissue was prepared for immunodiffusion tests using the methods described by Purcifull and Batchelor (4). The immunodiffusion medium consisted of 0.8% Noble agar, 0.5% sodium dodecyl sulfate (SDS), and 1% NaN_3. All wells were 6 mm in diameter, and each peripheral well was 5 mm from the center well. A 60-µl volume of reactant was added to each well, then the plates were incubated 4–24 hr in a humid chamber at 27 C before results were recorded. All antigen wells contained equal parts of tissue extract, water, and 3% SDS (v/v). Normal serum was used routinely as a control throughout this investigation.

The Clark and Adams (1) direct double-antibody sandwich method of enzyme-linked immunosorbent assay (DAS-ELISA) as performed by Zettler and Elliott (6) was used. Expressed leaf sap was diluted 1:9 (v/v) with extraction buffer before being added to the CCMV-Ch immunoglobulin-coated wells. The antiserum was prepared against non-degraded CCMV-Ch. The CCMV-Ch IgG conjugate concentration was 0.1 µg/ml (6).

Leaf extracts were negatively stained with 2% uranyl acetate and examined with a Hitachi 600 electron microscope for virus particles (6).

In one trial, plants of *N. benthamiana* were dusted with 0.22-µm-mesh Carborundum and manually inoculated with triturated chaya leaf extracts diluted in 0.2 M sodium phosphate buffer, pH 7.2.

RESULTS

Viral symptoms were not obvious in most of the cultivated chaya samples collected in Mexico. Although inconspicuous mosaic symptoms were occasionally seen in some of the specimens, nutritional disorders and insect damage often made diagnosis difficult. Cassava plants generally were in much better condition than chaya, but mosaic symptoms were not detected in any of the 25 cassava plants sampled in Mexico. Likewise, mosaic symptoms were not seen in any of the six wild *Cnidoscolus* or two wild *Manihot* samples collected in Mexico.

CCMV was detected serologically in 23 of the 33 cultivated chaya plants collected from various locations in the state of Yucatán (Fig. 1). Eight of the 10 samples from healthy plants were collected within a 10-km radius between Muna and Ticul (Fig. 1). DAS-ELISA results confirmed the SDS immunodiffusion results (Table 1). In SDS immunodiffusion tests, fused precipitin lines without spur formation were noted between CCMV-Ch antiserum and all of the infected chaya samples; in contrast, none of the infected samples reacted with CCMV-BPL antiserum. ELISA A_{405nm} values of 0.114–>2.000 were noted for samples that reacted positively in SDS immunodiffusion tests. Chaya samples that did not react in immunodiffusion tests yielded A_{405nm} values of only 0.000–0.066. Healthy chaya extracts used as controls gave A_{405nm} values of 0.006–0.020 in comparison to infected chaya controls, which gave A_{405nm} values of 0.101–0.372 (Table 1).

Flexuous rod-shaped virus particles were detected in negatively stained leaf extracts of 13 chaya samples that reacted positively in ELISA and SDS immunodiffusion tests (Table 1).

No evidence of CCMV or any other virus was detected in any of the 25 cultivated cassava or two wild *Manihot* plants assayed, nor was virus found in the six wild *Cnidoscolus* samples. Precipitin lines were not observed in SDS immunodiffusion tests with extracts of these plants and CCMV-Ch antiserum. Similarly, A_{405nm} values of only 0.000–0.038 were noted for nine cassava samples selected and tested by DAS-ELISA, whereas an average value of 0.783 was noted for a cassava sample used as a control that was known from previous studies to be infected with CCMV-Ch. No flexuous rod-shaped particles were seen in any of the three wild *Cnidoscolus* samples examined by electron microscopy.

Samples collected from six locations (Dzibichaltún, Libre Unión, Mérida, Muna, Piste, and Yaxcopoil) infected manually inoculated *N. benthamiana* seedlings. The foliar mosaic and distortion symptoms in these plants were like those described for CCMV-Ch (6). When tested in SDS immunodiffusion against CCMV-Ch antiserum, leaf extracts of these plants formed precipitin lines that fused with one another and CCMV-Ch antigen without spur formation. Precipitin reaction lines noted for leaf extracts of *N. benthamiana* sap-inoculated with CCMV-Ch were much stronger than those of the same isolates in chaya. Similar differences in antigen titers between *N. benthamiana* and chaya infected with Florida isolates of CCMV-Ch have been described previously (6). Precipitin lines between CCMV-Ch antiserum and *N. benthamiana* plants infected either with CCMV-Ch from Florida or any of the six Yucatán chaya virus isolates spurred over those of the cassava CCMV isolates provided by A. S. Costa and E. W. Kitajima, confirming our previous report. In reciprocal tests using CCMV-BPL antiserum, however, no reactions were observed for the chaya isolates tested (Fig. 2). Similar results were noted in our previous study, except weak precipitin lines were sometimes observed between CCMV-BPL antiserum and CCMV-Ch-infected *N. benthamiana* leaf sap (6).

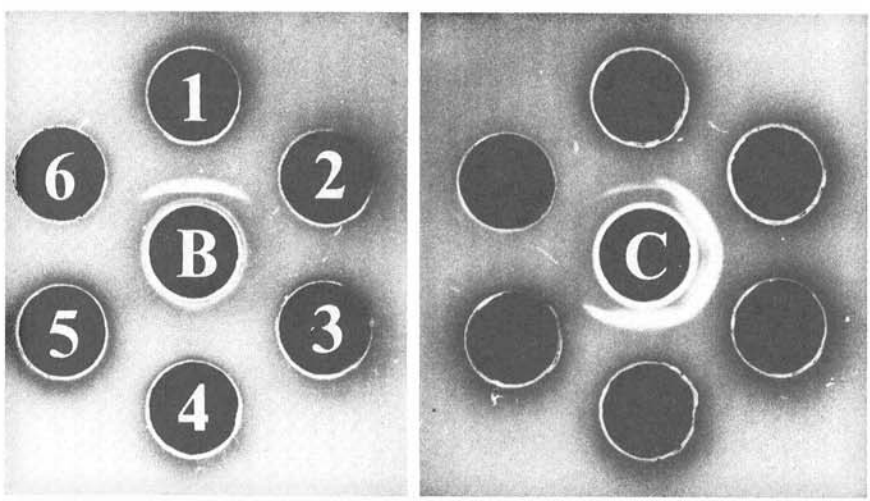

Fig. 2. Serological comparisons of cassava common mosaic virus isolates from chaya and cassava. Center wells contained (B) CCMV-BPL isolate antiserum and (C) CCMV-Ch isolate antiserum. Peripheral wells contained antigens (in SDS) as follows: 1 = cassava CCMV isolate provided by E. W. Kitajima in cassava, 2 and 3 = chaya CCMV isolates from Dzibichaltún and Mérida, respectively, in *Nicotiana benthamiana*, 4 = CCMV-Ch in *N. benthamiana*, 5 = healthy *N. benthamiana*, and 6 = healthy cassava.

DISCUSSION

This study shows CCMV to be widely distributed in cultivated chaya plants in the state of Yucatán; 70% of the 33 plants sampled were infected. The widespread distribution of CCMV in cultivated chaya is not surprising when we consider that this plant species must be propagated exclusively by vegetative means, that chaya has widespread popularity in Yucatán, and that, like most potexviruses, CCMV is readily transmitted manually. Although it is widespread in Yucatán, the significance of CCMV as a pathogen of chaya is not known. Other potexviruses such as potato X and cymbidium mosaic are considered serious pathogens, despite inducing inconspicuous symptoms in many of their respective hosts.

All isolates encountered in this study were serologically indistinguishable from the CCMV-Ch isolate described previously from chaya in Florida. The similarity of all the chaya isolates from Yucatán suggests they are from a common origin. Cassava, however, is not likely to be the source of CCMV inoculum for chaya in the Yucatán because of the absence of CCMV in any of the cassava samples tested in this study and serological differences noted between chaya isolates and cassava isolates from South America and Taiwan (6). Moreover, although currently grown experimentally at Uxmal, cassava is not widely grown in Yucatán, where maize is the primary starch source.

The CCMV-Ch-infected chaya plants studied in Florida (6) were from stock collected in Yucatán and subsequently grown in Mayaguez, Puerto Rico. It is probable that this isolate, like its host, originated from Yucatán on cultivated chaya plants. The indiscriminate exchange of chaya germ plasm from Yucatán could pose a threat to cassava plantings elsewhere. Because chaya isolates are significantly different serologically from those previously reported from cassava (6), they could be overlooked easily in programs that rely on relatively strain-specific indexing methods, such as DAS-ELISA (5). In this study, CCMV-BPL antiserum from cassava in Brazil failed to react in agar-gel immunodiffusion tests against any of the CCMV isolates found in Yucatán.

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