

Some Properties of a Cucumber Mosaic Virus Strain Isolated from Winged Bean in Florida

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ABSTRACT

Kuwite, C. A., and Purcifull, D. E. 1982. Some properties of a cucumber mosaic virus strain isolated from winged bean in Florida. *Plant Disease* 66:1071-1073.

A strain of cucumber mosaic virus designated CMV-WB was transmitted mechanically from leaves of winged beans showing chlorotic ring spots and mosaic symptoms to cowpea and other indicator plants. CMV-WB was aphid-transmitted in a nonpersistent manner. Virus purified from infected cowpea was used for antiserum production. CMV-WB was closely related serologically to Price's yellow strain of CMV and to a cowpea strain of CMV.

Winged bean, *Psophocarpus tetragonolobus*, has a high potential as a source of protein in the humid tropics (1,9). Several viruses have been reported to occur naturally in winged bean. These include a cucumovirus designated either psophocarpus ringspot mosaic virus (4) or winged bean ringspot mosaic virus (3); psophocarpus necrotic mosaic virus, which is rod shaped (3,4); cowpea mosaic virus (17); and yellow mosaic virus (18). To our knowledge, virus diseases have not been reported previously in winged bean in North America.

This report provides evidence that a mechanically transmissible virus isolated from naturally infected winged bean plants is a strain of cucumber mosaic virus.

MATERIALS AND METHODS

The virus used in this study was tentatively designated a winged bean strain of cucumber mosaic virus (CMV-WB). It was isolated from leaves showing mosaic and chlorotic ring spot symptoms collected from winged bean plants grown in field plots at the University of Florida in Alachua County in 1979.

Transmission. Inocula were prepared by grinding leaf tissue in 0.02 M potassium phosphate buffer, pH 7.5. Carborundum was added as an abrasive, and test plants were inoculated by being

rubbed with cheesecloth pads that had been dipped in inocula. Five to 10 plants of several species belonging to the families Amaranthaceae, Leguminosae, Solanaceae, Chenopodiaceae, and Cucurbitaceae were inoculated mechanically. Back-inoculations to cowpea (*Vigna unguiculata* 'Early Ramshorn' or 'Knuckle Purple Hull') were carried out 2-4 wk after inoculation to check for infectivity. For comparison, selected indicator plants were also inoculated with Price's yellow strain of CMV (13) and a cowpea strain of CMV (12).

The method of aphid transmission described by Zettler and Wilkinson (19) was adopted for this study. *Myzus persicae* (supplied by F. W. Zettler of the University of Florida) and *Aphis craccivora* (supplied by C. W. Kuhn of the University of Georgia) were tested as possible vectors. After a starvation period of 1-2 hr, the aphids were allowed to probe on infected cowpea or winged bean plants for 15-30 sec. They were then placed on noninfected cowpea or winged bean plants for 1 hr prior to being sprayed with malathion.

Purification. CMV-WB was purified from primary and trifoliolate leaves of cowpea plants collected 7-9 days after inoculation of the primary leaves. The method used was similar to method I reported by Francki et al (7), using sucrose density gradient centrifugation (7) or centrifugation in cesium sulfate gradients for further purification (E. Hiebert and D. Purcifull, unpublished).

Microscopy and spectrophotometry. To prepare crude leaf extracts for electron microscopy, tissues were diced in 2% potassium phosphotungstate, pH 6.5. Purified virus preparations were stained either with 2% potassium phosphotungstate or 2% uranyl formate and examined with a Hitachi H-600 electron microscope. Particle sizes were estimated by comparing projected electron micro-

graphs to micrographs of tobacco mosaic virus.

Virus concentrations were estimated by spectrophotometry using the extinction coefficient reported for CMV (7).

Serology. Antisera were produced to three types of CMV-WB preparations. One rabbit was immunized with untreated virus that was purified using cesium sulfate gradients; a second rabbit was immunized with virus that was purified in the same manner, except that the preparations were fixed with formaldehyde (5) prior to injection. A third rabbit was injected with sucrose gradient-purified virus treated with 0.1% sodium dodecyl sulfate (SDS) (15). The rabbits were injected two to four times over a period of several weeks, using antigen emulsified in adjuvant and a combination of toe pad and thigh muscle injection sites. General protocols and serum processing were as described elsewhere (14,15).

Antisera to either untreated or formaldehyde-treated CMV-WB were evaluated principally in a medium consisting of 0.8% agarose and 0.1% sodium azide (8), using crude leaf extracts prepared in water (2 ml of water per gram of tissue). Antisera to CMV-strains To and D (provided by J. C. Devergne) were also tested against CMV-WB using this system.

Antisera to SDS-treated CMV-WB, peanut stunt virus (ATCC PV-As 62), blackeye cowpea mosaic (11), and SDS-treated cowpea mosaic virus (11,15) were tested in 0.8% Noble agar, 0.5% SDS, and 1.0% sodium azide (14) using crude leaf antigens prepared in SDS (14,15).

Immunodiffusion test patterns and recording of results were as described previously (14,15). Freeze-dried reference antigens were used occasionally (14).

RESULTS

Host range. CMV-WB was readily transmitted mechanically from winged bean to the following plant species in which it caused the following symptoms: chlorotic ring spots and mosaic in winged bean; mosaics in cowpea, bean (*Phaseolus vulgaris* 'Bountiful' and 'Pinto'), *Pisum sativum* 'Little Marvel,' *Nicotiana benthamiana*, *N. clevelandii*, *Nicotiana* × *edwardsonii*, *N. glutinosa*, and *N. tabacum* 'Samsun' and 'Xanthi n.c.'; chlorotic lesions in inoculated leaves of *Chenopodium amaranticolor*; severe

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Florida Agricultural Experiment Journal Series 3669.

Accepted for publication 28 May 1982.

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0191-2917/82/11107103\$03.00/0

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mosaic and leaf puckering in *Gomphrena globosa*; and necrotic local lesions in *Vigna radiata* (from J. B. Quiot) and *V. radiata* lines PI 377166, PI 377167, PI 377168, PI 377020, PI 377021, and PI 377022 from the U.S. Southern Regional Plant Introduction Station. Cucumber (*Cucumis sativus* 'Long Maraicher') became infected but showed no distinct symptoms.

Aphid and seed transmission. CMV-WB was transmitted by *A. craccivora* from systemically infected cowpea leaves to 18 of 20 cowpea plants when five aphids were used per plant and to nine of 20 plants using one aphid per plant. *M. persicae* transmitted CMV-WB from cowpea to cowpea and winged bean and from winged bean to winged bean and cowpea.

CMV-WB was not transmitted through 135 seeds collected from infected winged bean plants in the field, nor through 37 seeds of cowpea (Knuckle Purple Hull)

harvested from plants inoculated and grown in the greenhouse.

Serology. Antisera to untreated and formaldehyde-treated CMV-WB reacted with aqueous crude sap extracts from leaves of cowpea and *Nicotiana × edwardsonii* infected with CMV-WB, using the agarose-azide medium of Gooding (8). The reactions consisted of a precipitin band located approximately midway between serum and antigen wells. These antisera had titers of 1/512 and 1/64, respectively; both sera also had a titer of 1/4 against sap extracted from healthy cowpea. Price's yellow strain of CMV and the cowpea isolate of CMV reacted with these CMV-WB antisera, and no spur formation was detected in comparisons with CMV-WB. CMV-WB antigen also reacted with Devergne's antisera to the To and D types of CMV, but the latter antigens were not available for comparison.

In SDS-immunodiffusion tests, the

antiserum to SDS-treated CMV-WB gave strong reactions against SDS-treated sap from infected *Nicotiana × edwardsonii* (Fig. 1) and from infected cowpea tissue. The three CMV isolates gave reactions of serological identity (eg, Fig. 1), whereas the bands formed by each isolate spurred over the reaction with peanut stunt virus (Fig. 1). The peanut stunt virus antiserum gave a weak homologous reaction (Fig. 1) but did not react with CMV-WB or the other two CMV isolates. The antiserum to SDS-treated CMV-WB had a titer of 1/16 against CMV-WB when tested by diluting antiserum with normal serum (14), and this antiserum did not react with sap from healthy plants. The antiserum to SDS-treated CMV-WB was used to detect the virus in infected winged bean plants grown in the field. Sap from leaves with pronounced symptoms gave strong reactions in SDS-immunodiffusion tests, whereas sap from leaves with mild symptoms gave much weaker immunoprecipitin lines. Some leaves from infected plants showed no symptoms, and extracts from them did not react serologically.

Properties of purified CMV-WB. Isometric particles about 31 nm in diameter were observed in electron micrographs (Fig. 2). Yields of virus averaged 30 mg/kg of tissue. The $A_{260/280}$ values of two preparations were 1.56. Purified virus induced symptoms in winged bean and cowpea similar to those induced when crude sap from CMV-WB infected plants was used as inoculum.

Stability of CMV-WB. Crude sap from systemically infected plants was infective for 24 hr at room temperature but not after 48 hr. Dried winged bean leaf tissue was still infective after storage over calcium chloride, under vacuum at 4 C, for 19 mo.

DISCUSSION

The evidence that the virus isolated from winged bean is a strain of CMV is based primarily on its close serological relationships to known CMV strains, particle morphology, and mechanical and aphid transmissibility (7). CMV-WB induced symptoms in winged bean that resembled closely those described for a seedborne cucumovirus in winged bean in the Ivory Coast (3,4).

The detection of CMV and serological comparisons of its strains by immunodiffusion tests have been complicated somewhat by the lability of the virus and by the occurrence of at least two distinct antigenic components (2,6,10). One component is much smaller and results in straight or slightly curved immunoprecipitin bands about midway between antigen and serum wells, whereas the other component (possibly intact virus) results in precipitin bands that curve around the antigen wells (2,10,16,20). Ziemiccki and Wood (20) reported that

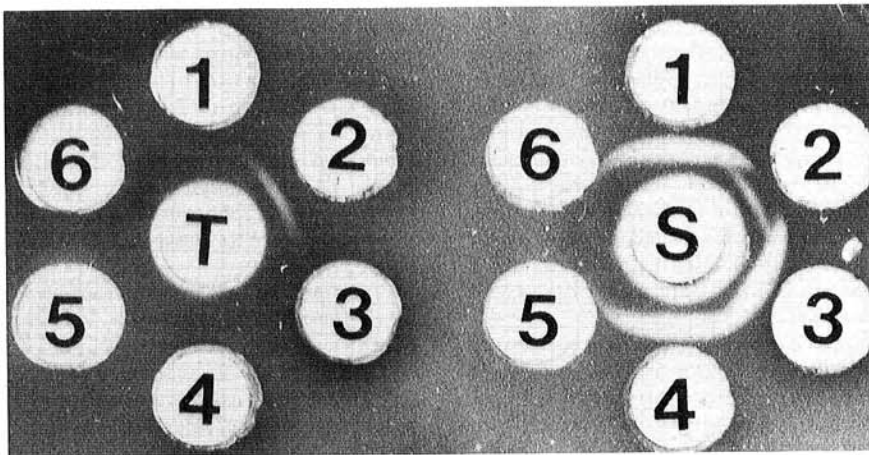


Fig. 1. Serological relationships of cucumber mosaic virus (CMV) isolates and peanut stunt virus (PSV). Immunodiffusion medium consisted of 0.8% Noble agar, 1.0% sodium azide, and 0.5% sodium dodecyl sulfate. The center wells were filled with undiluted sera: T = PSV antiserum PV-As 62 from the American Type Culture Collection; S = antiserum against the SDS-treated winged bean isolate of CMV (CMV-WB). The other wells were filled with SDS-treated crude leaf extracts from healthy or infected *Nicotiana × edwardsonii* as follows: 1 and 4 = CMV-WB; 2 = PSV; 3 = cowpea strain of CMV; 5 and 6 = healthy.

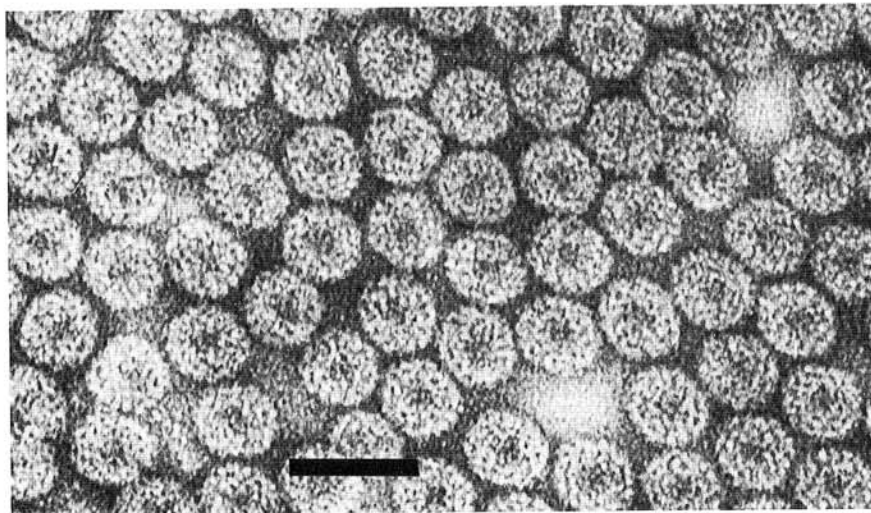


Fig. 2. Electron micrograph of a purified preparation of the winged bean isolate of cucumber mosaic virus stained with uranyl formate. Bar represents 50 nm.

the antigenic properties of low-molecular-weight antigen from SDS-treated CMV differ markedly from those of intact CMV. The SDS-immunodiffusion tests with CMV-WB and the other two CMV strains in our study gave reactions typical of those reported for low-molecular-weight antigens.

ACKNOWLEDGMENTS

We thank S. R. Christie and W. E. Crawford for assistance, E. Hiebert for suggestions concerning purification, and J. C. Devergne, C. W. Kuhn, J. B. Quiot, and F. W. Zettler for materials, as indicated in the text. The electron microscope used in this work was purchased, in part, using funds from National Science Foundation Grant PCM-7825524.

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