A Virus Related to Cucumber Mosaic Virus Isolated from Imported Ixora Plants

H. E. Waterworth and W. R. Povish

Research Plant Pathologist and Technician, Agricultural Research Service, U. S. Department of Agriculture, Glenn Dale, Maryland 20769.

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ABSTRACT

A virus was isolated from flowers of *Ixora* plants with mosaic imported from the Philippines. By host range, serological, and RNA characteristics, the virus was considered to be a strain of cucumber mosaic virus (CMV-Ix). However, in comparative studies, CMV-Ix was serologically distinct from CMV strains D, Q, and S, and differed in symptomatology on several host species, including cucumber, tomato, and datura. Antiserum, produced in a rabbit with formylated antigen, rose to a titer of 1:512 in successive bleedings. Virus particles maintained their structural integrity when stained with phosphotungstic acid without previous fixing.

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Additional key words: aphid transmission, physical properties.

Ixora spp. are subtropical evergreen woody shrubs with dense clusters (corymbs) of showy flowers ranging from white to shades of yellows and reds. Most of the 25 genera in Rubiaceae are not widely cultivated. Plants of *Ixora* imported from the Philippines in 1972 (P.I. 354123) showed a leaf mosaic, and upon indexing, a virus was isolated from the flowers of *Ixora* into *Nicotiana tabacum* L. 'Samsun'. This report describes the virus from *Ixora* that we have identified as an unusual strain of cucumber mosaic virus (CMV-Ix).

MATERIALS AND METHODS.—The antisera used were obtained from the American Type Culture Collection, from colleagues, or were produced in our laboratory. Our equipment, procedures, and special chemicals have been described (5). Cultures of CMV strains D, Q, and S were provided by J. M. Kaper. Peanut stunt virus (PSV) and tomato aspermy virus (TAV) were available also for host-range comparisons with the CMV-Ix.

Procedures for determining an experimental host range and physical properties, aphid transmissibility, and serological relationships have been described (5). Critical host ranges and symptoms were studied by inoculating diagnostic test species with the above strains of CMV and with CMV-Ix and comparing disease symptom development for 30 days.

Purification.—Fresh leaves of *Nicotiana tabacum* L. 'KY-35', inoculated two weeks earlier, were blended in two volumes (w:v) of cold 0.2 M sodium citrate buffer (pH 6.5) containing 0.02 M 2-mercaptoethanol. While blending, we added one-fourth volume of cold chloroform to the slurry. After centrifugation of the emulsion for 10 minutes at 10,000 *g*, we centrifuged the yellow aqueous phase at 105,000 *g* for 75 minutes. The amber-glassy pellets were covered with 0.05 M citrate buffer (pH 7.5) containing 2% Triton-X-100 and stored at 4 C. After 16 hours, the resuspended virus was centrifuged at 10,000 *g*, and the supernatant layered onto sucrose gradients. Purified virus was reconcentrated from fractions associated with the infectious-ultraviolet-absorbing region of the gradients. Yields were determined after analyzing a dilution of the final preparation in the spectrophotometer using a specific extinction coefficient of $E_{260}^{0.01} = 50.0$.

Antiserum production and serology.—A rabbit was immunized intramuscularly with formylated virus emulsified in Freund's incomplete adjuvant. Bleedings of 30 ml each were begun at the time of the fifth weekly injection. A similar immunization-bleeding regime was followed for the production of antiserum to CMV-S strain.

The virus from *Ixora* was identified by testing two concentrations of purified virus against several dilutions of antisera to 30 common spherical viruses.

RESULTS.—*Symptoms and host range.*—Leaves of the CMV-Ix source plants showed a conspicuous mosaic throughout the year (Fig. 1). However, neither growth nor flowering appeared to be affected. CMV-Ix differed

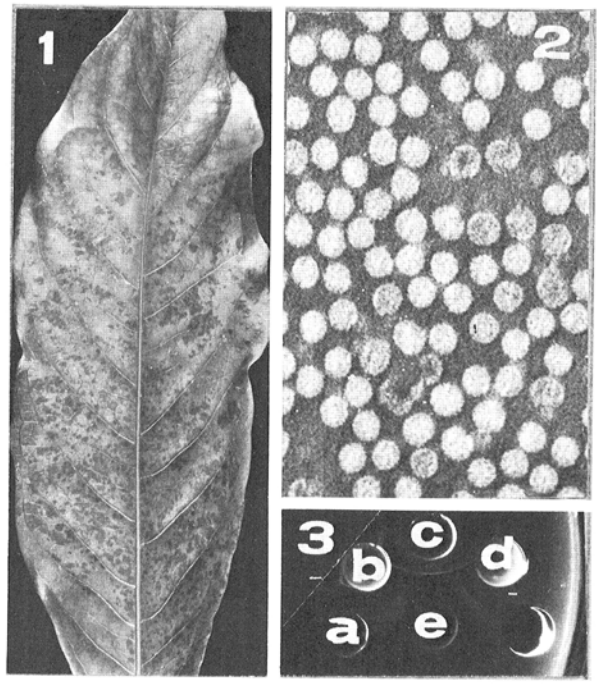

Fig. 1-3. 1) Leaf of *Ixora* with mosaic from which plant cucumber mosaic virus (CMV-Ix) was isolated. **2)** Purified CMV-Ix stained with 2% phosphotungstic acid (pH 7:1). **3)** Gel-diffusion serology with wells charged with purified (a) healthy plant sap, (b) CMV strain D, (c) CMV strain Ix, and (d) CMV strain S. Well E was charged with 1:16 dilution of antiserum to CMV-Ix.

from the other strains of CMV in that it did not incite systemic mosaic in *Cucumis sativus* 'Improved Long Green'. Rather, it caused a few large chlorotic spots in an occasional plant. Furthermore, CMV-Ix did not induce fern-leaf symptoms in *Lycopersicon esculentum* Mill. 'Rutgers'. It produced a mosaic in *Datura stramonium* L. which persisted, whereas plants infected with the CMV strains (D, Q, and S) recovered, and CMV-Ix caused a mosaic in *Capsicum annuum* L. 'California Wonder' that disappeared, whereas CMV-induced mosaics persisted. Moreover, it failed to induce pin-point lesions in *Vigna unguiculata* (L.) Walp. 'Blackeye' in most comparative tests, whereas strains of CMV produced numerous such lesions.

CMV-Ix also differed from common strains of CMV (4) in that it did not infect *Pelargonium zonale* (L.) L'Herit. ex A.T., *Calendula officinalis* L., *Brassica rapa* L., *Cynoglossum amabile* Stapf & Drumm., *Ipomoea batata* (L.) Lam., *Coreopsis grandiflora* Hogg, or *Tithonia rotundifolia* (Mill.) S. F. Blake. Furthermore, either CMV-Ix failed to incite symptoms in many species, or the symptoms were decidely less severe than those incited by CMV strains D and S. CMV-Ix did not infect mechanically inoculated plants of *Chrysanthemum morifolium* Ramat. 'Blue Ridge' or young seedlings of three peanut (*Arachis hypogaea* L.) cultivars, suggesting that CMV-Ix was not chrysanthemum aspermy or PSV, to which it was found to be serologically related. Physical properties and aphid transmissibility of CMV-Ix were similar to those of common CMV strains.

Purification, electron microscopy, and serology.—Yields of gradient-purified virus were 10-15 mg/100 g of tissue. The 260-280 ratio was 1.75. Purity was ascertained by electron microscopy, using Formvar-coated grids and staining the virus with 2% phosphotungstic acid adjusted to pH 7.1 with KOH. The average particle diameter was 24 nm (Fig. 2).

The antibody titer of CMV-Ix antiserum rose to 1:512 in successive bleedings. CMV-Ix was serologically related to PSV, TAV, and CMV. Antisera with homologous titers to PSV-T (1:128), TAV (1:128), and CMV (1:64) reacted with concentrated CMV-Ix when the antisera were diluted no more than 1:4, 1:4, and 1:16, respectively. However, CMV-Ix antiserum with a titer of 1:256 reacted with the concentrated antigens of CMV strains D and S (Fig. 3), PSV, and TAV, when the antiserum was diluted 1:256, 1:8, and 1:8, respectively. Hence, CMV-Ix was distantly related to PSV and TAV, and more closely related to CMV. CMV-Ix did not react with the antisera to any of the other viruses.

Ribonucleic acid.—When electrophoresed on polyacrylamide gels, RNA extracted from purified CMV-Ix resolved into four ultraviolet-absorbing components as have other strains of CMV RNA (2). However, CMV-Ix RNA had more of the B component and none of either the 00 [recently renamed "component 6" by Lot et al. (3)] or the C-1 components.

DISCUSSION.—The *Ixora* mosaic strain of CMV was decidedly different from CMV strains D, Q, and S in symptomatology among herbaceous hosts tested. It may have differed also in having particles 24 nm in diameter rather than the 30-nm diameter reported for other CMV strains (1). Furthermore, in contrast with the properties of some strains of CMV (1), CMV-Ix was purified by a simple procedure, was relatively immunogenic, and only a single curved band was observed in gel-diffusion serological tests. However, based on the existence of a four-component RNA, its physical properties, and its reciprocal serological relationship to CMV-S, we concluded that the *Ixora* virus should be considered a strain of CMV rather than a related virus.

LITERATURE CITED

1. GIBBS, A. J., and B. D. HARRISON. 1970. Cucumber mosaic virus. Plant Virus Description No. 1, Commonw. Mycol. Inst., Assoc. Appl. Biol., Hertsfordshire, Berks, England.
2. KAPER, J. M., and C. WEST. 1972. Polyacrylamide gel separation and molecular weight determination of the components of cucumber mosaic virus RNA. Prep. Biochem. 2:251-263.
3. LOT, H., G. MARCHOUX, J. MARROU, J. M. KAPER, C. K. WEST, L. VAN VLOTEN-DOTING, and R. HULL. 1974. Evidence for three functional RNA species in several strains of CMV. J. Gen. Virol. 22:81-93.
4. PRICE, W. C. 1940. Comparative host ranges of six plant viruses. Am. J. Bot. 27:530-541.
5. WATERWORTH, H. E., and J. M. KAPER. 1972. Purification and properties of carnation mottle virus and its ribonucleic acid. Phytopathology 62:959-964.