Cucumber Mosaic Virus from Cornflower in China

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ABSTRACT

Tien-Po, Rao, A. L. N., and Hatta, T. 1982. Cucumber mosaic virus from cornflower in China. Plant Disease 66:337-339.

A virus isolated from severely diseased cornflower (*Centaurea cyanus*) plants in China was identified as a strain of cucumber mosaic virus based on host range, particle morphology, serology, and nucleic acid analysis. Its serological relationship to several cucumber mosaic virus and tomato aspermy virus isolates present in Australia was determined.

Cornflower (*Centaurea cyanus* L.) is a popular ornamental garden plant in China. In a recent survey, 90% of these plants were found to be infected with a previously unidentified virus. Leaves of diseased plants exhibited severe mosaic and leaf deformation (Fig. 1A). We identified the causal agent as a strain of cucumber mosaic virus (CMV) that we will refer to as CMV-K. Isolation of CMV from cornflower had not been previously reported, although CMV is known to infect cornflower (6).

MATERIALS AND METHODS

Leaf tissue from diseased cornflower was ground in 0.01 M phosphate buffer, pH 7.0, and inoculated mechanically to *Nicotiana glutinosa* L. A pure virus culture was obtained by single lesion transfers in broad beans (*Vicia faba* L.) and was then maintained on *N. clevelandii* A. Gray. The virus was purified from systemically infected leaves of *N. clevelandii* as described by Peden and Symons (3) and centrifuged in 5–25% sucrose density gradients prepared in 0.02 M phosphate buffer, pH 7.5. Ribonucleic acid (RNA) was isolated from purified virus preparations by phenol-sodium dodecyl sulfate extraction (3) and analyzed by electrophoresis in 2% agarose gels (4). Thin sections for electron microscopy were prepared from young infected leaves with prominent symptoms and embedded in Epon, as described by Hatta and Francki (2). An antiserum to CMV-K was prepared in rabbits, and the serological tests were done by double diffusion in agar and by enzyme-linked immunosorbent assay (5).

Accepted for publication 12 November 1981.

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0191-2917/82/04033703/$03.00/0

RESULTS AND DISCUSSION

Symptomatology. Symptoms induced by CMV-K on various host species were typical of CMV (1). Systemic symptoms on *N. tabacum* L. cv. White Barley, *N. glutinosa, Nicotiana* × *edwardsonii* L., *N. clevelandii, Gomphrena globosa* L., and *Datura stramonium* L. were severe mosaic followed by leaf distortion. CMV-K was readily distinguishable from cucumovirus isolates present in Australia such as CMV-Q, CMV-X, CMV-U, CMV-M, CMV-T, tomato aspermy virus-V (TAV-V), and TAV-N (5) by its ability to infect maize (*Zea mays* L.) systemically. Like CMV-T and unlike all the other Australian strains of the virus, CMV-K induced reddish brown local lesions in broad beans (Fig. 1B) 5–7 days after inoculation.

Electron microscopy. Thin sections of maize leaves infected with CMV-K contained CMV-like particles in the cytoplasm, nuclei, and vacuoles (Fig. 1C). Tonoplast-associated vesicles characteristic of cucumovirus infection were also observed (2). Purified CMV-K preparations stained with uranyl acetate contained isometric particles measuring 29 nm in diameter (Fig. 1D). Most of the particles had dark stained centers in preparations fixed with glutaraldehyde (Fig. 1E), which are characteristic of cucumovirus (1).

Agarose gel electrophoresis. When CMV-K RNA preparations were subjected

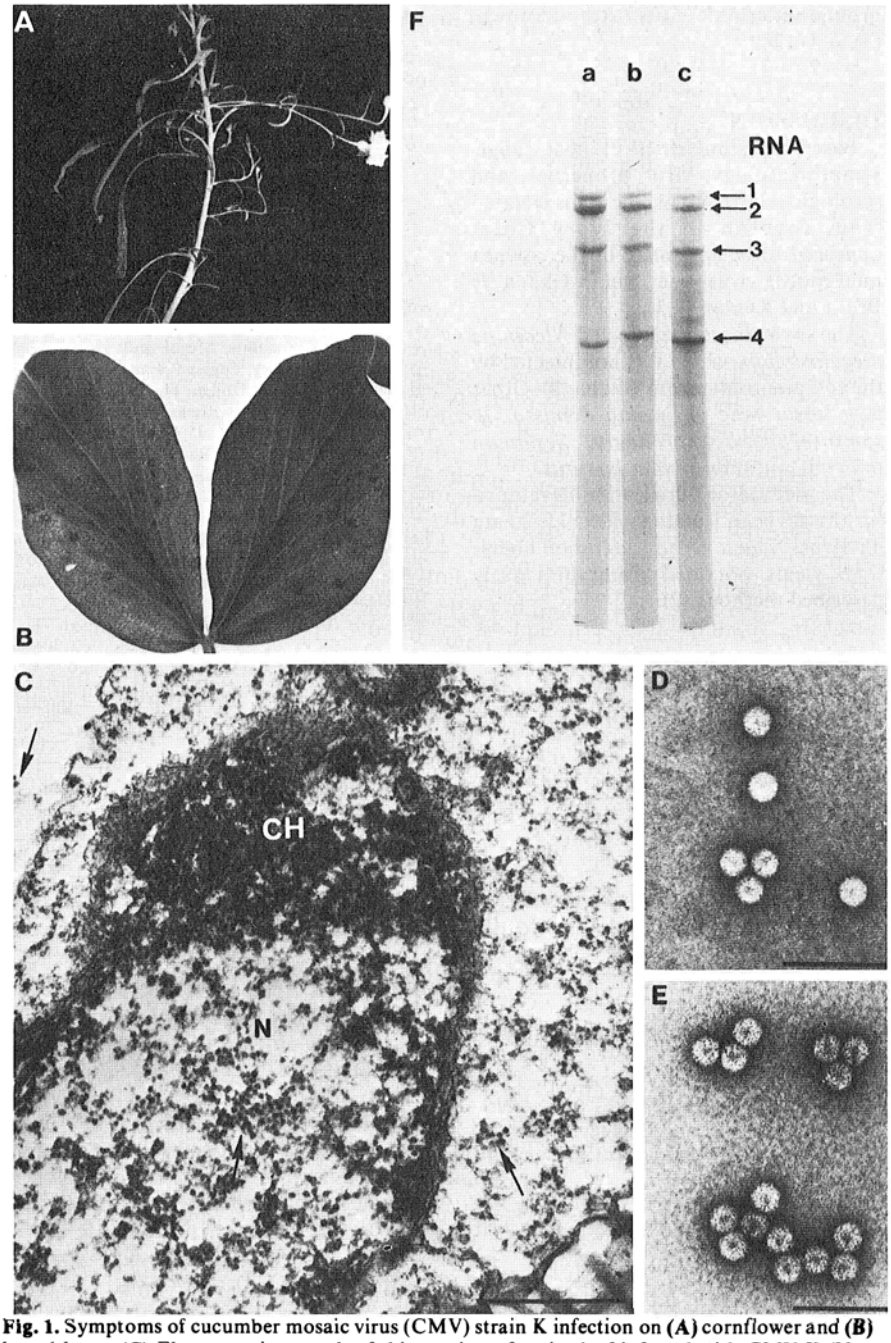

Fig. 1. Symptoms of cucumber mosaic virus (CMV) strain K infection on **(A)** cornflower and **(B)** broad bean. **(C)** Electron micrograph of thin section of maize leaf infected with CMV-K (N = nucleus, CH = chromatin). Arrows indicate viruslike particles. Bar represents 500 nm. **(D)** Purified preparations (unfixed) of CMV-K negatively stained with uranyl acetate. Bar represents 100 nm. **(E)** Glutaraldehyde-fixed preparations of CMV-K negatively stained with uranyl acetate. Bar represents 100 nm. **(F)** Two-percent agarose gel electrophoresis of ribonucleic acid preparations of **(a)** CMV-K, **(b)** CMV-T, and **(c)** CMV-K plus CMV-T.

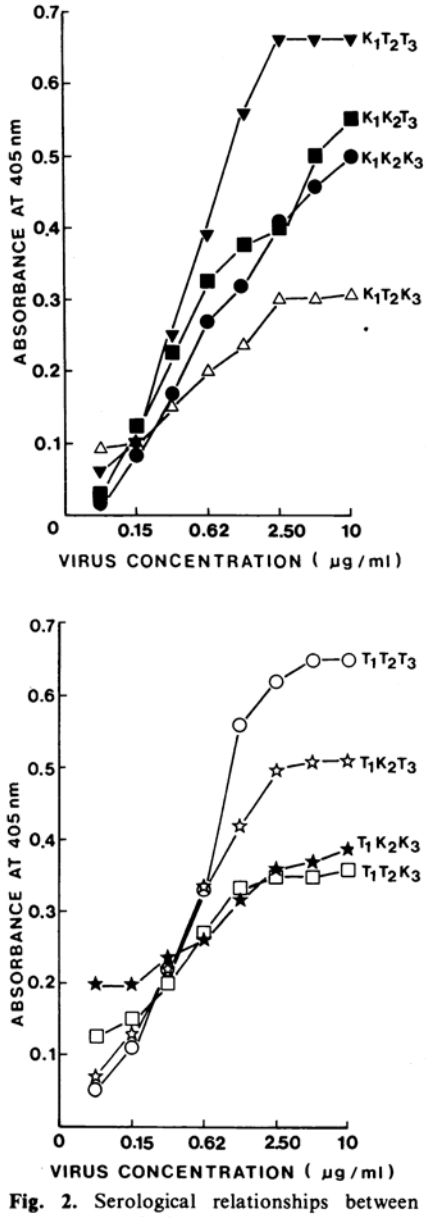

Fig. 2. Serological relationships between cucumber mosaic virus (CMV) strains K **(top)** and T **(bottom)** in enzyme-linked immunosorbent assay tests using homologous and heterologous combinations of coating and enzyme-linked antibodies. Letters refer to the virus strains used and numbers refer to the nature of the reagents (1 = coating antibody, 2 = antigen, and 3 = enzyme-linked antibody).

to electrophoresis in 2% agarose, they separated into four major RNA components (Fig. 1Fa) characteristic of CMV. Migration of the components was indistinguishable from those of CMV-T RNA (Fig. 1Fc).

Serology. In double diffusion tests, antiserum to CMV-K with a homologous titer of 1:256 reacted at dilutions of 1:128 against CMV-T and CMV-M; 1:64 against CMV-Q, CMV-X, and CMV-U; and 1:4 against TAV-V and TAV-N. Relationships between CMV-K and other CMV strains were also investigated in immunodiffusion tests using mixtures of anti-CMV-K and another CMV serum in one well and the two homologous antigens in separate wells (5). In these tests, absence of crossed precipitin lines or spurs with CMV-K and CMV-T and their antisera indicated a close serological relationship between the two viruses. In similar tests, spur formation (5) demonstrated antigenic differences between CMV-K and CMV-Q, CMV-X, CMV-U, and CMV-M. CMV-K and CMV-T were distinguished serologically (Fig. 2) by enzyme-linked immunosorbent assay using heterologous antibodies for coating and coupling enzyme, respectively (5). These results indicated that CMV-K was a typical strain of CMV relatively closely related to strains of the virus isolated in Australia.

ACKNOWLEDGMENTS

We are grateful to R. I. B. Francki for discussion and correction of the manuscript, L. Wichman for line drawings, and D. W. Talfourd for supply and maintenance of plants. The first author acknowledges support from the University of Adelaide Distinguished Visitor's Fund, the Australia-China Council, and the Adelaide University Research Grant Scholarship Fund. The third author acknowledges support from a Commonwealth Special Research Grant of the Department of Primary Industry.

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