Unusual Strain of Cucumber Mosaic Virus Causing Flower-Breaking Symptoms in Wild Violets

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ABSTRACT

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Wild violets (*Viola* spp.) showing flower-breaking symptoms were found in many locations in northwestern Arkansas. A virus with some properties similar to cucumber mosaic virus (CMV) was isolated from infected flowers. Particle size and shape, sedimentation coefficient, and molecular weights of protein subunits and four RNA components were similar to those of other CMV strains. The virus, designated CMV-Vi, differed from the other CMV strains in its relatively narrow host range, high instability, and serology. In Ouchterlony double-diffusion tests, CMV-Vi reacted only with its homologous antiserum and not with those of four CMV strains. Other strains of CMV reacted with CMV-Vi antiserum. Five *Viola* spp., *V. papilionaceae, V. sagittata, V. sororia, V. triloba,* and *V. viarum*, were found naturally infected with CMV-Vi.

Additional key words: cucumoviruses, electron microscopy

Wild violets (*Viola* spp., Violaceae) with flower-breaking symptoms are commonly observed in northwestern Arkansas. Mechanical inoculation of sap extracts from symptom-bearing flowers readily induced viruslike symptoms on various plant species. This suggested that a virus may induce the flower-breaking symptoms. Further studies indicated that these symptoms were indeed caused by a virus identified as a strain of cucumber mosaic virus (CMV). This CMV strain, however, has some properties that are significantly different from known strains (1,4). This paper describes the properties of the virus designated CMV-Vi.

MATERIALS AND METHODS

Wild violets with flower-breaking symptoms were collected from many locations in northwestern Arkansas. Studies on the properties of CMV-Vi were carried out using an isolate from the flowers of *V. papilionaceae* Pursh.

Physical properties were determined as described by Stevens (10). Cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata* 'TVu 612') was used as local lesion host. Virus infectivity was lost very rapidly when extracted from *Nicotiana megalosiphon* H.&M. leaf tissue. Procedures commonly used for CMV purification (1,2) yielded very low and often noninfective virus. High yields of virus, however, were obtained using infected, frozen violet petals. These were ground with 5 ml of 0.01 M sodium phosphate buffer, pH 7.0, per gram of petals, the extract centrifuged at 5,000 *g* for 10 min, and the supernatant subjected to one high-speed centrifugation (80,000 *g* for 90 min). Pellets were suspended in 0.01 M sodium phosphate buffer, pH 7.0, and centrifuged in 0.2–0.7 M sucrose gradients prepared in the same buffer in a Beckman SW-27 rotor at 25,000 rpm for 150 min. Purified virus was centrifuged at 32,000 rpm in the An-D rotor in Beckman Model E analytical ultracentrifuge with Schlieren optics. The sedimentation coefficient was determined using Markham's graphical method (7). Coat protein subunits were analyzed by electrophoresis in 7.5% SDS polyacrylamide gel using a vertical slab-gel apparatus (6). Protein subunits of tomato aspermy virus (TAV), peanut stunt virus (PSV), and CMV-D were used for comparisons. The RNA was extracted with SDS-$NaClO_4$ (13), denatured using glyoxal (8), and analyzed by electrophoresis in 1% agarose gel, using a horizontal slab-gel apparatus. Comparisons were made with RNAs of CMV-D and PSV. For electron microscopy, purified virus was stained with 2% uranyl acetate and placed on a Formvar-coated grid. Thin sections of infected leaf and violet petals as well as comparable healthy tissues were prepared for electron microscopy as described by Kim et al (5). Antiserum for CMV-Vi was prepared following procedures described previously (12). Ouchterlony double-diffusion tests were prepared with 1% agarose in 0.01 M sodium phosphate buffer, pH 7.0. Antisera for CMV-Vi, CMV-D, CMV-C, CMV-Y, CMV-AB, TAV-B, TAV-A, and PSV-W (obtained from H. A. Scott) were reacted with purified preparations of CMV-Vi, CMV-D, CMV-C, CMV-Q, CMV-14, TAV-A, and PSV-W (supplied by I. B. Ahmad) in different combinations.

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RESULTS

Natural hosts. Five *Viola* spp. were found naturally infected with identical isolates of CMV-Vi. They were *V. sororia* Willd., *V. papilionaceae* Pursh., *V. sagittata* Ait., *V. viarum* Pollard, and *V. triloba* Schwein. Symptoms on all of these *Viola* spp. consisted of flower breaking without leaf symptoms. Virus was mechanically transmitted only from flowers but never from leaf tissue of all five violet species.

Host range. Plant species susceptible to CMV-Vi are shown in Table 1. Symptoms on *V. papilionaceae, Nicotiana megalosiphon, V. unguiculata* (L.) Walp. subsp. *unguiculata* 'TVu 612,' and *N. tabacum* L. 'NC 95' are shown in Figure 1. Plants not susceptible to CMV-V were *Capsicum annuum* L. 'California Wonder'; *Cucumis sativus* L. 'Model'; *Commelina*

diffusa Burm.; *Cucurbita maxima* Dcne. 'Ranger'; *Datura stramonium* L.; *Lycopersicon esculentum* Mill. 'Traveler'; *Phaseolus vulgaris* L. 'Pinto,' 'Black Valentine,' 'Viva Pink,' 'Scotia,' and 'Black Turtle Soup'; *P. aureus* Roxb.; *N. tabacum* L. 'Havana 38'; *N. glutinosa* L.; *N. silvestris* Speg. & Comes; *Solanum melogena* L. 'Black Beauty'; and *Zea mays* L. 'Funks G-4740.' All plants were back-inoculated to cowpea TVu 612.

Physical properties. Virus was extremely unstable in infected leaf extracts of any of the susceptible hosts (Table 1). Infectivity of leaf sap of infected *N. megalosiphon* and *N. clevelandii* was lost in less than 20 min at room temperature. Antioxidant agents such as cysteine-HCl, 2-mercaptoethanol, and thioglycollic acid failed to extend infectivity. In sap extracted from petals of infected *V. papilionaceae*, longevity in vitro was between 24 and 48 hr, the thermal inactivation point between 55 and 60 C, and the dilution end point between 10^{-3} and 10^{-4}. After 2 yr of storage at −20 C, infected flower samples were still infective.

Purification. Purification from infected *N. megalosiphon* leaf tissue was not satisfactory. Virus purified from infected violet petals gave high yields of up to 20 mg of virus per 10 g of petals. Infectivity of purified preparations was lost after 5 days at 4 C in 0.01 M sodium phosphate buffer, pH 7.0. Infectivity was lost faster (within 48 hr) when purified virus was suspended in EDTA and/or sodium borate buffer at different molarities and pH.

Sedimentation coefficient. Purified preparations of CMV-Vi sedimented as one single component with a value of approximately 98 S.

Protein. Protein of CMV-Vi comigrated as a single polypeptide together with CMV-D, TAV, and PSV proteins.

RNA. Glyoxal-denatured RNA of CMV-Vi, CMV-D, and PSV showed four similar electrophoretic components.

Electron microscopy. Thin sections of infected petals showed numerous isometric virus particles in the cytoplasm, with particles occasionally arranged in crystalline arrays (Fig. 2). Relatively few viruslike particles were seen in infected leaf tissue. Viruslike particles were not observed in healthy tissues. Icosahedral particles about 28 nm in diameter were evident in negatively stained purified preparations.

Serology. Preparations of purified CMV-Vi (3 mg/ml) and extracts from infected petals reacted with its homologous antiserum (titer 1:4) but did not react with any of the heterologous antisera. Purified CMV-Vi, CMV-Q, CMV-C, and CMV-14 (3 mg/ml) reacted with CMV-Vi antiserum. Spur formation was evident when any of these CMV strains was reacted side by side with CMV-Vi (Fig. 3).

DISCUSSION

Although a stable strain of CMV has been reported from commercial violets and pansies in California (9), CMV-Vi apparently is quite distinct. The instability of CMV-Vi, particularly under buffer and pH conditions suitable for most CMV strains, is unusual for a cucumovirus. Although sodium phosphate was the best buffer, loss of infectivity gradually occurred in this buffer also. The relatively narrow host range of CMV-Vi is a possible consequence of the instability of the virus. An interesting observation was the occurrence of large numbers of virus particles in the petal tissue. In contrast, virus particles were seen only in small numbers in symptomless leaf tissue. Inhibitors present in *Viola* spp. leaf extract combined with a low virus concentation and virus instability may be the reason for the failure to mechanically transmit the virus from that tissue. An antiserum to this CMV strain had a titer of only 1:4,

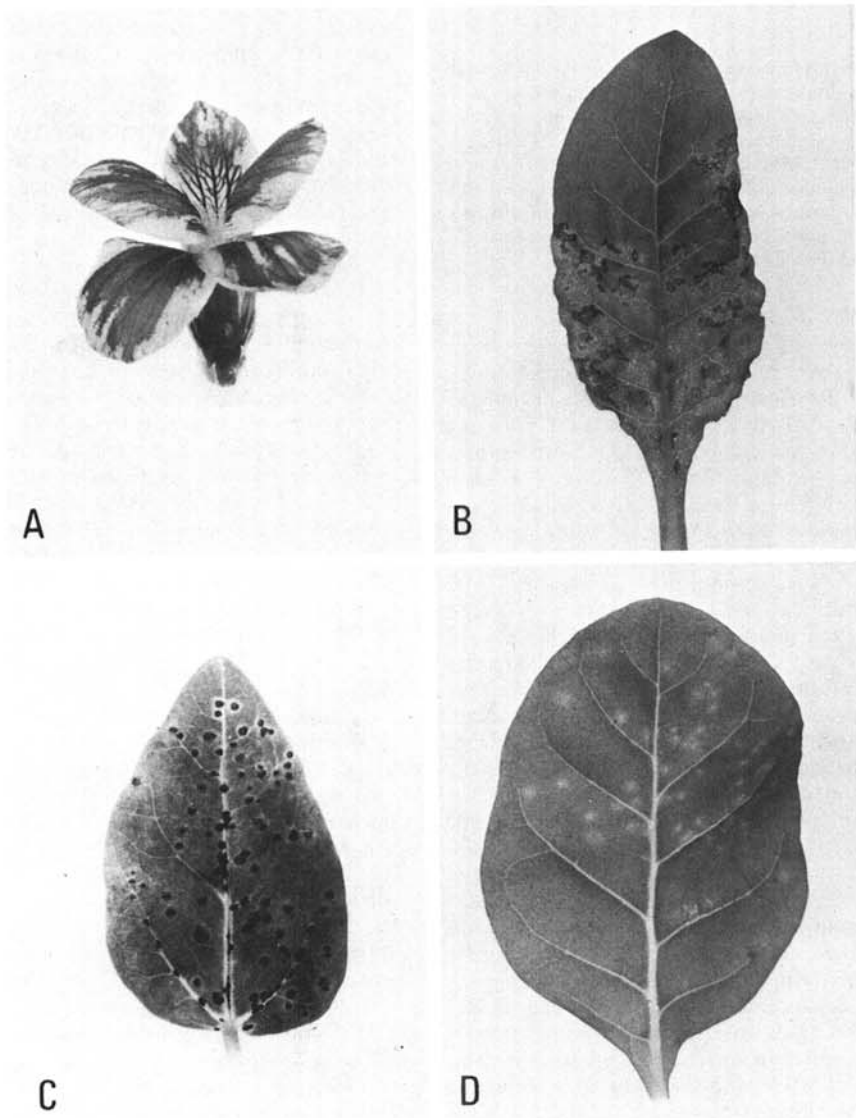

Fig. 1. Symptoms induced on different hosts by cucumber mosaic virus strain-Vi **(A)** Flower breaking on *Viola papilionaceae*, **(B)** systemic necrosis on *Nicotiana megalosiphon*, **(C)** necrotic local lesions on *Vigna unguiculata* subsp. *unguiculata* 'TVu 612,' and **(D)** chlorotic local lesions on *N. tabacum* 'NC 95.'

Table 1. Plant species susceptible to cucumber mosaic virus strain-Vi after mechanical inoculation[a]

Plant species	Symptoms[b]
Chenopodium quinoa	Chll
Gomphrena globosa 'Mixed Colors'	Chll
G. globosa 'Orange'	Lrs
Nicotiana benthamiana	S
N. clevelandii	Srs, Smo
N. debneyi	S
N. megalosiphon	Sn, Smo
N. tabacum 'NC 95'	Chll
Vigna unguiculata (L.) Walp. subsp. *unguiculata* 'TVu 612'	LLnl
'Crimson,' 'Chinese,' 'Monarch'	LLns
Viola cornuta	Sn
V. odorata	Sn, Smo
V. papilionaceae	Fb

[a] Inoculum consisted of an extract from infected flowers of *V. papilionaceae*.

[b] Chll = chlorotic local lesions, Srs = systemic ringspot, S = symptomless host, Smo = systemic mottle, Sn = systemic necrosis, Fb = flower breaking, Lrs = localized ringspots, LLnl = necrotic local lesions large, and LLns = necrotic local lesions small.

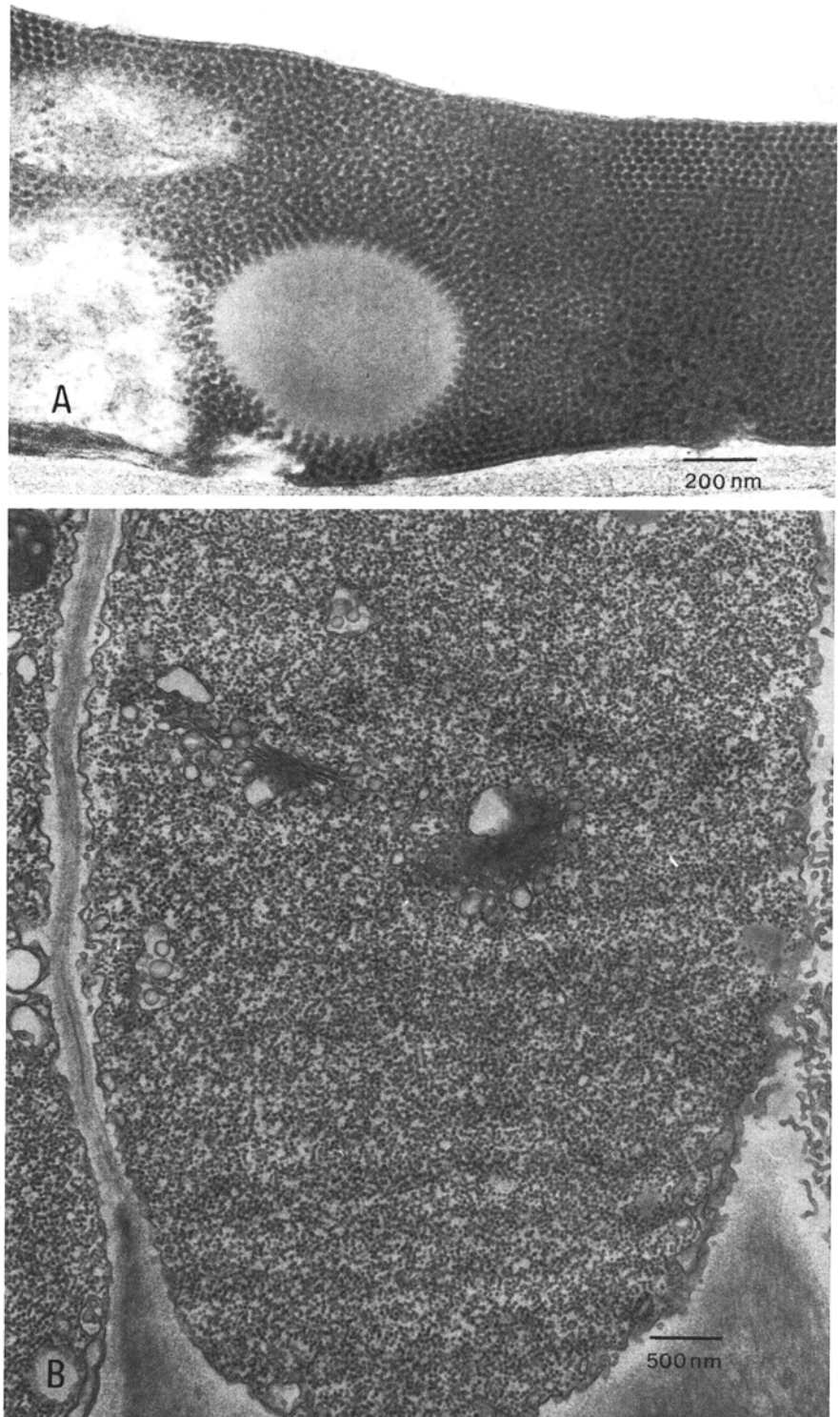

Fig. 2. Electron micrographs of thin sections of *Viola papilionaceae* petals infected with cucumber mosaic virus strain-Vi. **(A)** Crystalline arrays and **(B)** masses of viral particles in the cytoplasm.

suggesting that it is a poor immunogen. The failure to obtain a serological reaction with CMV-Vi and several heterologous antisera is a characteristic not uncommon among cucumoviruses (4). This, together with the spur formation with other CMV strains when CMV-Vi antiserum was used, confirms the uniqueness of this CMV strain. Other properties, such as particle size and shape, sedimentation coefficient, and molecular weight of protein and RNA components, were very similar to those reported for other CMV strains (2,4).

This CMV strain is apparently indigenous to northwestern Arkansas. Reports of wild violets with variegated flowers (2,11) suggest that CMV-Vi may well be widespread in other regions of the United States.

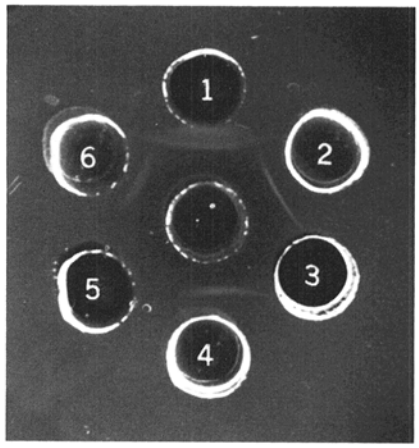

Fig. 3. Ouchterlony double-diffusion test showing the reactions of different cucumoviruses with antiserum for cucumber mosaic virus strain-Vi (CMV-Vi). Center well contains antiserum to CMV-Vi. Peripheral wells contain preparations of purified virus: 1 = CMV-Vi, 2 = CMV-D, 3 = peanut stunt virus-W, 4 = CMV-Q, 5 = tomato aspermy virus-B, and 6 = CMV-C.

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