Identification of a Cucumber Mosaic Virus Strain from Naturally Infected Peanuts in China

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ABSTRACT

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A virus isolated from an infected peanut seedling grown from seed collected in Jing County, Liaoning Province, China, was identified as a strain of cucumber mosaic virus, designated CMV-CA, on the basis of biological, physical, and chemical properties, serology, and particle morphology. This strain infected 31 of 36 species tested in six families. The longevity in vitro was 6–7 days, the thermal inactivation point was 55–60 C, and the dilution end point was 10^{-2}–10^{-3}. The aphid *Macrosiphum euphorbiae* transmitted the virus in a nonpersistent manner. Seed transmission in peanut was about 1.3%. Virus particles were spherical (28.7 nm in diameter). An antiserum against CMV-CA was produced with titers of 1/256 and 1/64 in microprecipitin and gel diffusion tests, respectively. Serologically, CMV-CA was indistinguishable from CMV-D and distinct from CMV-S. There was a more distant serological relationship between CMV-CA and tomato aspermy virus but no serological relationship between CMV-CA and peanut stunt virus strains E, W, or T. The $S_{20,w}$ value of the particle and molecular weights of CMV-CA coat protein and of the four RNAs agreed with reported values for CMV.

Viruses have been prevalent in some parts of the peanut (*Arachis hypogeae* L.) growing areas of northern China since 1976, when virus diseases caused severe losses in peanut (24). A survey by the first author in July 1981 revealed that the incidence of virus-diseased plants was as high as 100% in some peanut fields on the farm of the Agricultural Research Institute of Da-Lian District and as high as 60% in fields of Jing County in Liaoning Province. Symptoms of the disease were characterized by chlorotic spots on young emerging leaves, chlorosis of young expanded leaves that were smaller and rolled, mosaic or mottling of some leaves, and moderate stunting of the plant (one-half to two-thirds the height of healthy plants). A virus was isolated from a seedling with mosaic symptoms that had been grown from seeds collected in 1981 from infected plants in a field in Jing County. This paper presents the identification of the virus from naturally infected peanut plants in China as a strain of cucumber mosaic virus, designated CMV-CA (China *Arachis* strain).

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MATERIALS AND METHODS

The virus was subcultured from a local lesion on *Datura stramonium* L. to *Nicotiana clevelandii* Gray and maintained in *N. clevelandii*. All plants infected with CMV-CA were maintained in a growth chamber at 28 C with 12-hr photoperiods.

Infected plant tissues were ground in 0.03 M sodium phosphate buffer, pH 8.0, and rubbed on corundum-dusted leaves of six plants of each species tested. After 1 mo, inoculated leaves and uninoculated upper leaves from each species were assayed for virus infection by inoculation to *Chenopodium amaranticolor* Coste & Reyn.

Aphid transmission tests were done with *Macrosiphum euphorbiae* (Thomas) by conventional methods for a nonpersistent virus: acquisition and test plant *N. clevelandii*, preliminary fasting 2 hr, acquisition feeding 2 min on healthy or infected plant, and test feeding 24 hr with five aphids per plant.

Seed transmission frequency was determined with peanut seeds (Xu Zhou 68-4) collected in China from naturally infected plants. Seedlings were checked for infection by visual observation, back-inoculation to test plants, and serological tests.

N. clevelandii was used as a source of infected tissue for in vitro property tests and for purification. In vitro property tests were done by grinding 1 g tissue in 5 ml 0.03 M sodium phosphate buffer, pH 8.0.

The purification procedure was a modification of that reported by Francki (8) for CMV. After precipitation by polyethylene glycol, the pellets were resuspended in 0.03 M sodium phosphate buffer, pH 7.0, instead of the 0.05 M sodium borate, 0.05 M EDTA, pH 9.0, buffer used by Francki. After one cycle of differential centrifugation, further purification was achieved by a 4-hr density gradient centrifugation at 95,000 *g* in 10–50% glycerin diluted with the sodium phosphate buffer.

Purified virus was negatively stained with 2% uranyl acetate, pH 2.7, for electron microscopy. The diameters of 200 virus particles were measured. A grating carbon replica of 2,160 lines per millimeter served as a magnification standard.

For antiserum preparation, the virus was dialyzed overnight against 0.2% formaldehyde and subsequently dialyzed against the sodium phosphate buffer. A rabbit received an intravenous injection (0.75 mg virus) followed by a subcutaneous injection (1 mg virus in 1 ml of buffer emulsified with Freund's complete adjuvant, 1:1) and two injections with emulsification in Freund's incomplete adjuvant at 2-wk intervals. Titers of the antisera to CMV strains CA, D, S, and CI (cowpea isolate) were determined by both microprecipitin and gel diffusion tests. Serological relationships were determined by gel double-diffusion and enzyme-linked immunosorbent assay (ELISA) serological tests (19). Tomato aspermy virus (TAV) and antiserum were supplied by G. Gooding; PSV-W antiserum was from G. I. Mink and the PSV-W culture was from G. I. Mink via S. A. Tolin; the CMV-S and CMV-D cultures were received from J. M. Kaper; antisera against CMV-S (PVAS-242a) and CMV-D (PVAS-260) were from the American Type Culture Collection, Rockville, MD; antiserum to the Blencoe tomato isolate, designated CMV-Bt here, was from H. A. Scott; and antiserum to CMV-CI was from our laboratory.

Molecular weight of virus coat protein was analyzed by electrophoresis in 12.5% polyacrylamide gels by the method of Laemmli (15). The following proteins were used as standards: α-lactalbumin (mol wt 14,400), soybean trypsin inhibitor (mol wt 20,100), carbonic anhydrase (mol wt 30,000), ovalbumin (mol wt 43,000), bovine serum albumin (mol wt 67,000), and phosphorylase b (mol wt 94,000).

CMV-CA RNA was treated with proteinase K overnight at room temperature, heated at 60 C for 5 min, cooled, and extracted by the phenol-

hydroxyquinoline:*m*-cresol procedure (20). RNA electrophoresis procedures and molecular weight standards were reported previously (23). Analytical ultracentrifugation was in AMV buffer (16).

RESULTS

Host range of CMV-CA. CMV-CA infected 31 of 36 plant species in six families. Plants systemically infected showed mosaic or mottle symptoms (except those with other symptom types in parentheses)—Amaranthaceae: *Gomphrena globosa* L., *Vinca rosea* L., and *Zinnia elegans* Jacq.; Chenopodiaceae: *Beta vulgaris* L. and *Spinacia oleracea* L. (stunt); Cucurbitaceae: *Cucumis sativus* L. 'Chicago Pickling' (latent); Gramineae: *Zea mays* L. 'Golden Cross Bantam' (stunt and wilt); Leguminosae: *A. hypogaea, Canavalia ensiformis* (L.) DC. (stunt), *Cassia occidentalis* L., *Dolichos lablab* L., *Phaseolus lunatus* L. 'Henderson Bush', *P. vulgaris* L. 'Bountiful,' 'Pinto,' and 'Topcrop,' *Pisum sativum* L. 'Dwarf Grey Sugar' and 'Perfected Wales' (systemic necrosis and wilt), *Trifolium incarnatum* L., *Vicia faba* L. (streak), and *Vigna unguiculata* subsp. *unguiculata*; Solanaceae: *Lycopersicon esculentum* L. (latent), *N. benthamiana* Domin., *N. clevelandii, N. glutinosa* L., *N. megalosiphon* Heurck & Meull., *N. occidentalis* Wheeler, *N. tabacum* L. 'Burley 21,' *Physalis floridana* Rydb., and *Solanum melongena* L. Plants infected only on inoculated leaves showed local lesions—Chenopodiaceae: *Chenopodium album* L., *C. amaranticolor, C. quinoa* Willd.; Leguminosae: *Phaseolus aureus* Roxb.; Solanaceae: *Datura stramonium* L. Plants not infected were Gramineae: *Triticum aestivum* L.; Luguminoseae: *Glycine max* (L.) Merr. 'Bragg,' 'Davis,' and 'Jackson,' *Trifolium hybridum* L., *T. pratense* L., and *T. repens* L.

The following are symptom descriptions on some plant species used as indicator or propagation plants or that might be useful as diagnostic plants for CMV-CA:

A. hypogeae showed symptoms similar to those in field-infected peanut plants. Young expanding leaves had chlorotic spots and showed rolling 4–5 days after inoculation. Later, these leaves showed mosaic or mottle patterns with vein-clearing (Fig. 1), and plants were about two-thirds the height of healthy plants.

C. amaranticolor developed numerous chlorotic pinpoint lesions, some of which had white centers, on inoculated leaves 2 days after inoculation (Fig. 2). No systemic infection occurred.

Dolichos lablab showed chlorotic local lesions 2 days after inoculation. Later, systemic yellow areas developed along the veins of young expanding leaves (Fig. 3).

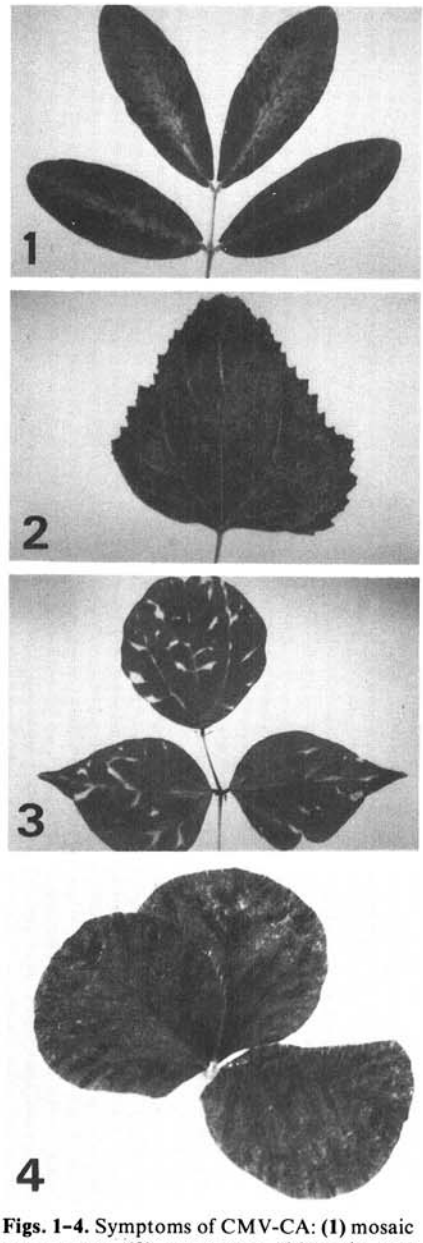
N. clevelandii produced chlorotic local lesions 3–4 days after inoculation. Later, young leaves showed systemic mosaic.

Trifolium incarnatum exhibited a systemic mosaic on young leaves 1 wk after inoculation (Fig. 4).

Cucumis sativus and *Lycopersicon esculentum* were systemically infected without symptoms.

Properties of CMV-CA in vitro. In sap extracted from *N. clevelandii* plants infected for 7 days, CMV-CA infectivity was lost at dilutions between 10^{-2} and 10^{-3} by heating for 10 min to between 55 and 60 C and by storage at 28 C between 6 and 7 days.

Transmission of CMV-CA. The virus was transmitted to five of 10 *N. clevelandii* plants by *Macrosiphum euphorbiae*. All control plants remained symptomless. Of 242 peanut seedlings grown from seeds collected in Jing County, three (1.2%) with leaf mosaic were found to be infected with CMV-CA. Of 295 peanut seedlings from seeds collected on the Da-Lian Institute's farm, four (1.4%) seedlings were infected with CMV-CA.



Figs. 1–4. Symptoms of CMV-CA: **(1)** mosaic on peanut, **(2)** numerous chlorotic and necrotic pinpoint lesions on inoculated leaf of *Chenopodium amaranticolor*, **(3)** systemic yellow spots along veins on *Dolichos lablab*, and **(4)** mosaic on *Trifolium incarnatum*.

Purification and properties of purified CMV-CA. Extracts made in 0.5 M sodium citrate buffer, pH 6.5, had greater infectivity and gave higher virus concentrations than extracts made in 0.3 or 0.1 M sodium phosphate buffers, pH 7.0. The $A_{260/280}$ of the virus was 1.50. Virus yields of 2–3 mg/100 g tissue were obtained.

Electron microscopy of CMV-CA. Spherical particles with dark-stained centers characteristic of cucumovirus were seen by electron microscopy. Negatively stained particles averaged 28.7 nm in diameter. Phosphotungstic acid (2%, pH 7) disrupted unfixed virus particles.

Serological tests. CMV-CA antiserum had homologous titers of 1/256 and 1/64 in microprecipitin and gel diffusion tests, respectively. This antiserum had similar titers with CMV strains D and CI in most tests but had somewhat lower titers with CMV-S (Table 1). In gel double-diffusion tests, the CMV-CA antiserum reacted with all CMV strains and CMV-CA reacted with antisera to CMV strains D, S, CI, and Bt. The precipitin lines to CMV strains CA, D, or CI coalesced, whereas spurs developed between CMV-S and strains CA, D, or CI when antisera to CMV strains CA, D, S, and CI were used (Figs. 5–7). This indicated that CMV-CA was close to CMV-D and CI but distinct from CMV-S serologically.

CMV-CA produced a weak reaction with antiserum to TAV with a strong TAV spur over that of CMV-CA. CMV-CA did not react with antisera to PSV strains E, W, or T (Fig. 8).

In ELISA with CMV-CI antiserum, CMV-CA and CMV-D reacted near the homologous reaction level, whereas CMV-S reacted weakly and PSV did not react. With PSV-E antiserum, CMV-CI reacted weakly compared with the homologous reaction level, whereas CMV strains CA, D, and S gave no reaction (Table 2).

Molecular weight of the CMV-CA coat protein subunit and RNAs. Polyacrylamide gel electrophoresis of the CMV-CA coat protein subunit gave a molecular weight of 26,000, which is close to reported values (12,26).

Polyacrylamide electrophoresis in nondenaturing conditions of the RNAs of CMV-CA revealed four principal components with molecular weights of 1.16×10^6, 1.05×10^6, 0.81×10^6, and 0.39×10^6. Although not in close agreement with RNA molecular weight values considered to be highly accurate

Table 1. Homologous and heterologous serological reactions among cucumber mosaic virus (CMV) strains

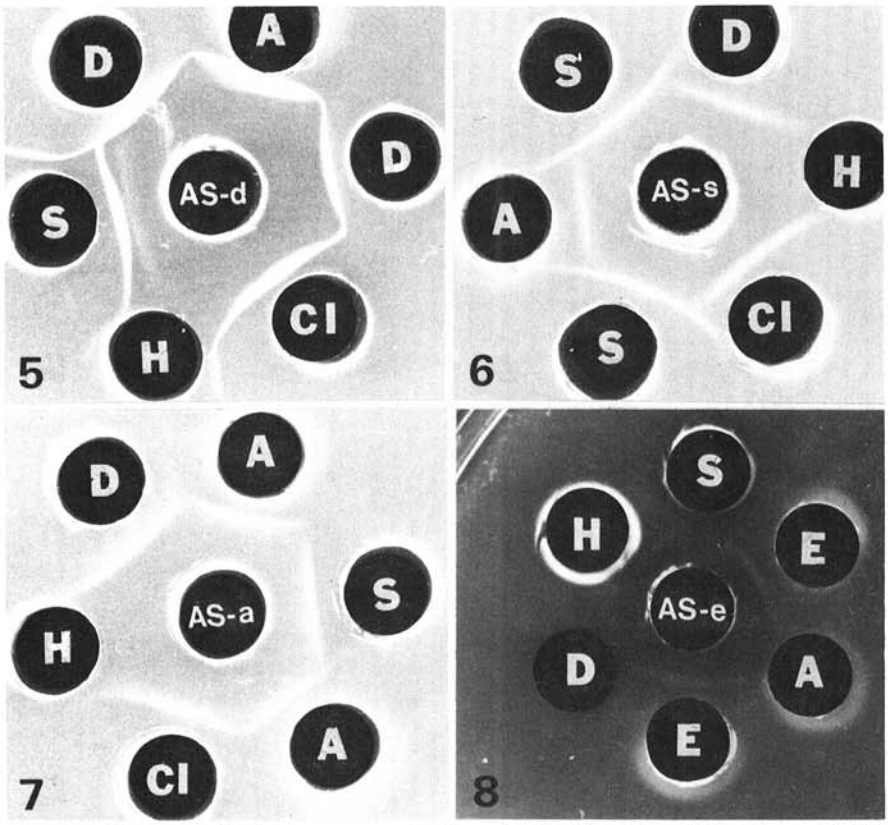
Antiserum	Antigen			
	CMV-CA	CMV-D	CMV-CI	CMV-S
CMV-CA				
MPT[a]	256[b]	256	···	64
GDT	64	64	64	32
CMV-D				
MPT	1,024	1,024	···	512
GDT	256	256	128	64
CMV-CI				
MPT	···	···	···	···
GDT	32	16	32	8
CMV-S				
MPT	128	128	···	256
GDT	64	64	64	256

[a] Abbreviations: MPT = microprecipitin test: GDT = gel diffusion test. Starting antigen concentrations were 0.25 mg/ml for MPT and 0.5 mg/ml for GDT.
[b] Reciprocal values of titers. ··· = Not tested.

Table 2. Reaction (A_{405}) of cucumber mosaic virus (CMV) strains and peanut stunt virus (PSV) with antisera to CMV-CI and PSV in ELISA[a]

Experiment no.	Antiserum	Antigen					Healthy tissue
		CMV-CI	PSV	CMV-CA	CMV-D	CMV-S	
1	CMV-CI	1.74	0.12	1.34	···	···	0.19
	PSV	0.63	1.19	0.26	···	···	0.17
2	CMV-CI	1.80	0.21	0.66	0.90	0.33	0.19
	PSV	0.67	1.75	0.32	0.40	0.38	0.29

[a] Coating antibody was used at 5 μg/ml. Antibody conjugated with alkaline phosphatase was used at 0.625 μg/ml for CMV-CI and 2.5 μg/ml for PSV. Antigen was infected leaf material ground in 0.02 M phosphate, 0.15 M sodium chloride, 0.003 M potassium chloride, pH 7.3, and 0.05% Tween 20 (1 g tissue in 9 ml).



Figs. 5–8. Gel double-diffusion tests among CMV strains CA, S, D, CI, and PSV-E using **(5–7)** 1 mg/ml of purified antigens and **(8)** infected plant sap. AS-d = antiserum to CMV-D, AS-s = antiserum to CMV-S, AS-a = antiserum to CMV-CA, AS-e = antiserum to PSV-E, A = CMV-CA, S = CMV-S, D = CMV-D, CI = CMV-CI, E = PSV-E, and H = healthy tissue. The medium was 0.8% agarose with 0.1% sodium azide in 0.03 M sodium phosphate buffer, pH 7.0.

(12), these values fall within the range of values in the literature (ie, 9) and confirm that CMV-CA has four RNA components similar to many other strains with little or no RNA-5.

Sedimentation properties of CMV-CA. CMV-CA sedimented with a symmetrical single boundary in 0.03 M sodium phosphate buffer containing 0.001 M Na_4EDTA, pH 7.2. At a concentration of 1 mg/ml, its $S_{20,w}$ was 93.6 S, which is in the range of reported values (8,17).

DISCUSSION

Several legume crops naturally infected by CMV have been reported (1–3,6,10,11,13,14,18,21,22,25,27); however, there is no report of natural infection of peanut by CMV, and in fact, noninfection of peanut has been a diagnostic characteristic of CMV (7,12).

CMV-CA infected several species in the Leguminosae, as do several legume isolates of CMV (1,6,10). In contrast, CMV-CA infected but caused no symptoms in cucumber or tomato and infected cowpea, French bean, and peanut systemically although most CMV isolates either produce local lesions or do not infect these plants (8). Devergne and Cardin (4) assigned a number of CMV isolates to two serotypes. CMV-CA belongs to the DTL serotype. In contrast to some CMV isolates in the DTL serotype (5), CMV-CA did not react with PSV-E antiserum. Like some CMV strains, CMV-CA had a distant serological relationship with TAV (4).

Serological tests precluded any possibility that CMV-CA was a strain of PSV although CMV-CA had some similarities to PSV in host range, eg, they both showed wide host ranges in legume plants. There were some differences between CMV-CA and PSV in symptomatology and host range. PSV usually caused severe stunting of peanut plants and veinclearing followed by a mosaic on cowpea, whereas CMV-CA induced only moderate stunting of peanut and a faint mosaic on cowpea. CMV-CA failed to infect red and white clovers even though both are susceptible to PSV.

Added in galley: *Phaseolus mungo* was a useful indicator in field surveys because it develops brown local lesions with no systemic infection when inoculated with CMV-CA. Many, but not all, field isolates of CMV reacted weakly with PSV-E antiserum.

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