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NATURAL INFECTION OF MUNGBEAN (VIGNA RADIATA) WITH CUCUMBER MOSAIC VIRUS

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ABSTRACT

Cucumber mosaic virus caused serious damage to mungbean (Vigna radiata) plants grown at the University of Missouri, Agronomy Research Center at Columbia during the past several years. The affected plants showed pronounced mosaic, leaf deformities, stunting and, occasionally, flower abortion and pod distortion. The causal virus was transmitted readily with sap inoculation and was seedborne in mungbeans (0.51%). It was acquired and transmitted by the cowpea aphid (Aphis craccivora) during brief probes. Identity of the causal virus was confirmed with host range, electron microscopy, and serology.

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During the past 6-7 years, symptoms indicative of viral infection have been observed on a large number of mungbean (Vigna radiata) strains grown at the University of Missouri, Agronomy Research Center at Columbia, Missouri. These mungbean strains showed varying degrees of disease severity ranging from extremely susceptible to highly resistant, based upon a visual scoring procedure (11, 12). Although the susceptible mungbean strains showed pronounced mosaic, vein-banding, leaf puckering, stunting, and reproductive abnormalities, the resistant strains appeared normal.

One resistant mungbean strain (PI 271401) and five susceptible strains (PI 246130, PI 201868, PI 368288, PI 368299, and PI 368307) were grown in the field during 1974 under insect-proof cages and an unprotected control plot. Typical disease symptoms appeared on the susceptible strains in the unprotected plot, but plants grown under the insect-proof cages showed no evidence of disease. This observation suggested that the causal virus was being transmitted by a vector. The cowpea aphid (Aphis craccivora) was found colonizing the field-grown mungbeans and was confirmed subsequently as a vector for this virus (11). Evidence presented in this report confirms the identity of this mungbean virus as cucumber mosaic virus (CMV).

MATERIALS AND METHODS

The virus isolate used in this study was recovered as follows: Field-collected A. craccivora were allowed an access period for 24 hr on a mungbean (PI 201868) plant showing typical disease symptoms. These aphids were then transferred and maintained (24 hr) on caged 7-day-old healthy mungbean seedlings (PI 201868) after which the aphids were killed with fumigation. Symptoms appeared on several mungbean plants in 15-20 days following exposure to the aphids. The virus was subsequently transmitted with sap-inoculation from these mungbean plants and maintained on mungbean (PI 201868) and Gomphrena globosa.

Colonies of virus-free aphids were established in a greenhouse by serially transferring single nymphs at 5-day intervals to healthy mungbeans (PI 201868). Diseased mungbean plants served as the inoculum source in the insect transmission tests. Aphids were starved for 4 hr at 25° C, before the acquisition feeding. All inoculation feedings were done on healthy mungbean (PI 201868) seedlings. Experiments concerning virus : vector relationship were repeated 3-4 times by using a total of 40-65 test plants. Viral transmission efficiency (%) was calculated from the number of plants infected relative to those exposed to the aphids.

Inoculum for mechanical transmission was prepared by homogenizing leaf tissue in ice-cold 0.067 M sodium phosphate buffer, pH 7.0, and filtering the extract through cheesecloth. Celite (50 mg/ml) was added to the extract and the test plants were rubbed with a cotton applicator dipped in the inoculum. In experiments on physical properties of the virus, assays were performed on the primary leaves of mungbean (PI 271401) which was found to be a local-lesion host. Back inoculations were performed on mungbean PI 271401 and G. globosa plants.

Antisera against CMV were obtained from the American Type Culture Collection (PVAS 88), Rockville, Md., and Dr. R. G. Grogan, University of California, Davis. Serologic tests were done with the Ouchterlony double diffusion method. Identical results were obtained with both these antisera when reacted against the mungbean virus. Leaf dip samples stained with uranyl formate were examined with a Jeol 100B electron microscope.

Seeds harvested from field-infected mungbeans (PI 368307) were planted in a greenhouse under insect-free conditions. Sap extracted from plants showing symptoms was assayed on mungbean PI 271401 and G. globosa, and also reacted against CMV antiserum.

RESULTS

Aphid transmission: By using eight aphids per test plant, the transmission efficiency of the mungbean virus by A. craccivora was approximately 40%, whereas with single aphids it varied from 15-20%. After a pre-acquisition starvation period of 4 hr, single aphids were able to acquire or inoculate plants during a 30-second probe. Prolonging the probing period to 1 or 5 minutes had no additional effect on the transmission efficiency. These results suggest that the mungbean virus is carried on the aphid's stylet, an observation which is consistent with the other reports on the mode of CMV transmission by aphids (1, 3).

Host range and Symptomatology: Results on the experimental host range of the mungbean virus and symptoms induced on representative hosts are presented in Table 1 and Figure 1 (A-F). Presence of the virus in these plants was confirmed with back inoculation to the differential hosts. None of the uninoculated plants maintained as controls in the host range study showed evidence of any viral infection.

The mungbean virus was poorly transported in cucumber cvs. London, Model Pickling, and Straight Eight. This was indicated by the fact that upon inoculating the cotyledonary leaves, symptoms appeared on the first one to three true leaves only. No symptoms developed and the virus was not recovered beyond the fourth to fifth leaf on these plants. Munger and Newhall (7) made a similar observation on mosaic-infected 'Chinese Long' cucumbers. The progression of symptom development by this virus in tomato and tobacco plants was also slow. In other systemic hosts, it was transported rapidly to the newly opened leaves.

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Table 1. Host range and symptoms induced following sap inoculation with the mungbean isolate of cucumber mosaic virus.

Plant Species	Symptoms Induced
AMARANTHACEAE	
Gomphrena globosa	Occasional red lesions, mosaic, leaf puckering
CHENOPODIACEAE	
Chenopodium amaranticolor	Chlorotic local lesions
C. quinoa	Mosaic
CUCURBITACEAE	
Cucumis sativus 'London'	Mottle, ring spots
C. sativus 'Model Pickling'	Mottle, ring spots
C. sativus 'Straight Eight'	Mottle, ring spots
LEGUMINOSAE	
Lens culinaris	Mosaic
Phaseolus lunatus 'Fordhook Bush'	Mosaic, yellowing of leaves
P. lunatus 'Henderson Bush'	Mosaic
P. lunatus coccineus 'Butterbean'	Mosaic, yellowing of leaves
P. acutifolius 'Tepary 4' U.S.A.	Local lesions
P. acutifolius PI 321637	Local lesions
P. acutifolius PI 200749	Local lesions
P. vulgaris 'Bountiful'	Mosaic, yellowing and puckering of leaves
P. vulgaris 'Kentucky Wonder'	Mosaic and leaf yellowing
Pisum sativum 'Alaska'	Mosaic and yellowing
P. sativum 'Fordhook Wonder'	Mosaic and yellowing
Trifolium repens	Mosaic and leaf chlorosis
Vigna radiata PI 201868	Mosaic, veinal chlorosis and necrosis, leaf distortion, flower abortion, stunting
PI 271401	Small, discrete local lesions
PI 362322	Large, expanding lesions
PI 377362	Large, expanding local lesions
V. unguiculata 'Black Eye'	Mosaic, leaf yellowing and stunting
SOLANACEAE	
Lycopersicon esculentum 'Marglobe'	Mild mottle
Nicotiana tabacum 'Java'	Mild mosaic, occasional vein-banding
N. tabacum 'Xanthi-nc'	Mild mottle

Mungbean strain PI 271401 proved to be a reliable local lesion host for the mungbean CMV isolate. Discrete necrotic spots developed on the primary leaves following inoculation with no evidence of systemic spread to the trifoliate leaves. Inoculation of the trifoliate leaves, similarly, resulted in the appearance of only local lesions.

The following plant species grown in the close proximity of mungbeans in the field during 1976 also became infected with the virus: G. globosa, Chenopodium amaranticolor, Phaseolus vulgaris, Lens culinaris, Pisum sativum, Lathyrus odoratus and Lycopersicon esculentum. Presence of the virus in these plants was confirmed with back inoculation on mungbean PI 271401.

No symptoms developed nor was the virus recovered from the directly inoculated or non-inoculated leaves of the following plant species: Brassica carinata, B. campestris, B. oleracea capitata, Citrullus lanatus, Cucumis melo, Cucurbita pepo, Avena sativa, Festuca arundinacea, Hordeum vulgare, Triticum aestivum, Cicer arietinum, Medicago sativa, and Glycine max (cvs. Dare, Hill, and Clark 63).

Preliminary properties: In crude mungbean or G. globosa leaf extracts, the virus was inactivated when heated at 55-60°C for 10 min. The dilution end-point and longevity in vitro of the virus were, respectively, 1 : 500 to 1 : 1000, and 48 hr (22-24° C). Leaf dip preparations from the diseased plants showed presence of isometric (30 nm, diameter) particles (Fig. 1G), whereas no such particles were observed in the comparable healthy leaves.

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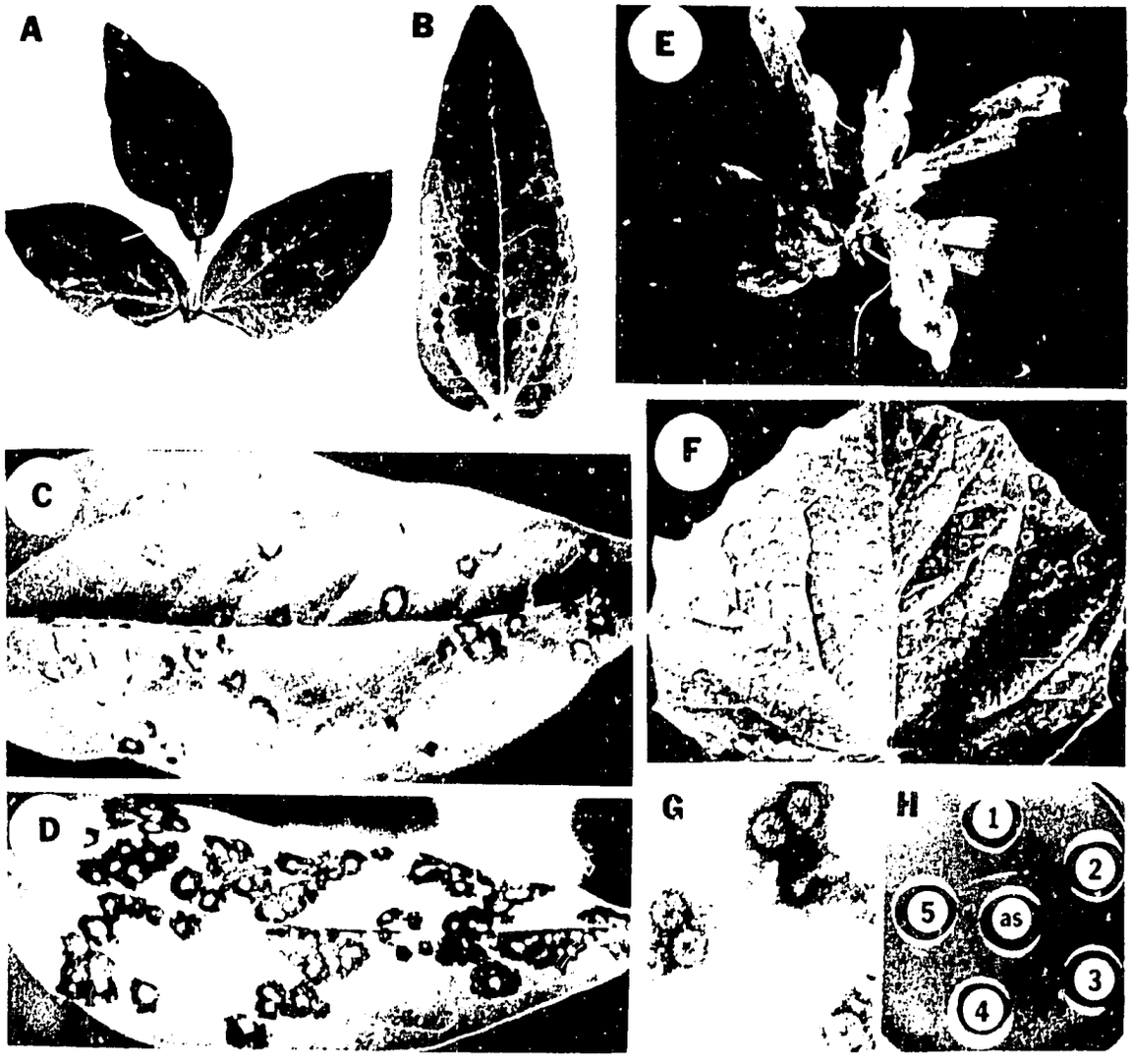

FIGURE 1. Symptoms induced on representative plants (A-F), particle morphology (G), and serologic identity of the mungbean virus (H). Mottling, veinal chlorosis and necrosis on trifoliate mungbean (PI 201868) leaves (A); local lesions on the primary leaves of mungbean PI 271401 (B); mungbean PI 377362 (C); and mungbean PI 362322 (D). Mosaic and leaf distortion on Gomphrena globosa (E); mottling and ringspots on cucumber cv. Model Pickling (F). Isometric particles (magnification, 160,000) in the leaf dip preparations of diseased G. globosa plants (G). Serologic reaction using antiserum against CMV-C (ATCC, PVAS 88) and sap extracted from diseased and healthy G. globosa plants (H); wells 1, 2, and 3 were charged with, respectively, 1 : 3, 1 : 6, and 1 : 12 dilution of sap from diseased plants, while wells 4 and 5 contained, respectively, 1 : 3 and 1 : 6 dilution of healthy plant extract. The central well (as) contained the antiserum (dilution, 1 : 5).

In double diffusion tests, when reacted against Grogan's CMV antiserum or CMV-C antiserum (ATCC, PVAS 88), extracts from the mungbean virus-infected plants produced distinct precipitin bands, while no reaction occurred with comparable extracts from healthy plants. Fig. 1H shows results of such a test using CMV-C antiserum and sap from diseased and healthy G. globosa plants. Distinct precipitin reaction was evident with 1 : 3 and 1 : 6 diluted sap from diseased plants, while with 1 : 12 dilution, a faint reaction was observed; no reaction occurred with 1 : 3 or 1 : 6 diluted sap from healthy plants. No precipitin reaction was observed when such extracts were reacted with normal serum or antisera prepared against southern bean mosaic, bean pod mottle, cowpea mosaic, or tobacco ringspot viruses.

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Seed transmission: Out of 1313 seedlings raised from seed harvested from infected mungbeans, eight showed typical symptoms (transmission, 0.61%). Presence of CMV-M in these plants was confirmed with test inoculations and serology.

DISCUSSION

Evidence based upon host range, preliminary properties, particle morphology, and serology employing antisera from two different sources, confirm the identity of our mungbean virus as CMV. Field infection of mungbean with CMV has been reported also from Iran (4, 5). Although the relationship between our mungbean CMV isolate and other CMV strains (1, 2, 3, 4, 8, 9, 10) is not clear, slight differences appear to exist. For instance, the mungbean CMV isolate differs from other CMV strains because (i) of a very restricted host range among cucurbits, (ii) induction of mild symptoms on solanaceous plants, (iii) production of systemic symptoms on cowpea, and (iv) lower dilution end-point and thermal tolerance. A culture of this virus has been deposited with the American Type Culture Collection.

Local lesions induced by the mungbean CMV isolate on mungbean PI 271401 resemble those caused by the common bean mosaic virus on mungbean, 'Oklahoma 12' (6). These two viruses are different, however.

Although Kaiser, et al. (4, 5) failed to observe seed transmission of CMV through lentil (Lens esculenta), Phatak (8) reported appearance of mild mosaic and chlorosis in a few seedlings raised from mungbean seed obtained from Iran; presence of CMV in these seedlings was confirmed with particle morphology and host range. In our studies, the efficiency of the mungbean CMV transmission through mungbean seed was 0.61%.

The identity of plants which might serve as the local overwintering reservoirs for the mungbean virus remains to be established fully. Mungbeans grown in one season about 30 km from the Agronomy Research Center showed no visible signs of viral infection. The only perennial species naturally infected with this virus in the immediate vicinity of mungbean plots at Columbia, Missouri, was white clover (Trifolium repens). These plants and the seedborne virus are probably the initial source of the viral inocula for local dissemination by the cowpea aphid and other aphid species. Because several plant species found susceptible to mungbean CMV infection with sap-inoculation in the greenhouse were also found infected in the field, it appears that A. craccivora is a natural vector of this virus.

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