Natural Infection of Mungbean by Bean Common Mosaic Virus

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ABSTRACT

A seed-borne, aphid-transmitted virus was found infecting mungbeans (*Vigna radiata*) in various regions of Iran. The virus produced deformation, puckering, rolling, blistering, and mosaic symptoms on the foliage of virus-infected mungbeans. The pathogen was seed-borne in mungbean (8 to 32%) and common bean (*Phaseolus vulgaris*) (approx. 7%). The mungbean virus adversely affected growth and yields of mungbean, especially when infection occurred before pod set. Yields from 11 mungbean lines infected from seed were reduced by 31 to 75%. Two mungbean lines were highly resistant to the virus in field trials at Karaj, Iran. The virus was transmitted in a stylet-borne manner by several aphid species, including *Aphis craccivora, Acyrthosiphon pisum,* and *Acyrthosiphon sesbaniae*. Electron micrographs of negatively stained leaf-dip preparations showed that infection was associated with flexuous particles approx. 750 nm in length. From host range studies, symptoms, seed and vector transmission, serology, and particle morphology, the mungbean virus was identified as a strain of bean common mosaic virus.

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In 1968, a seed-transmitted virus disease of mungbean [*Vigna radiata* (L.) Wilczek (synonym: *Phaseolus aureus* Roxb.)] in Iran was described by Kaiser et al. (3). The disease was called mungbean mosaic virus and appeared to be related to bean common mosaic virus (BCMV). This virus is the most important disease affecting mungbeans in Iran. It is found in most areas where the crop is cultivated.

A similar seed-borne virus disease affecting urd bean or black gram [*Vigna mungo* (L.) Hepper (synonym: *Phaseolus mungo* L.)] was described by Shahare and Raychaudhuri (6) in India in 1963. The virus, which also infected mungbean, resembled BCMV in its physical properties and host range. Nene (5) found the virus, which he renamed mosaic mottle, to be widely distributed in northern India. In greenhouse screening trials, he found resistance to the virus in one urd-, and two mungbean, lines.

The present investigation was initiated to study various properties of the Iranian mungbean virus (M-BCMV), including host range, transmission by seeds and insects, effects on yield, and varietal resistance.

MATERIALS AND METHODS.—Diseased and healthy mungbeans collected in different pulse-growing regions of Iran were triturated in distilled water, 0.01 M phosphate buffer (pH 7.0), or 1.0% K_2HPO_4 (pH 7.0). The sap was applied to Carborundum-dusted 44 μm (320-mesh) leaves of young, healthy indicator plants with sterile Q-tips, or by hand (thumb and forefinger). To avoid mixed viral infections, isolates of M-BCMV were purified by serial transfer of the etiologic agent from single local lesions that developed on primary leaves of mungbean. Isolates of M-BCMV were propagated on bean (*Phaseolus vulgaris* L. 'Bountiful'). Plants included in the host-range studies were indexed on healthy Bountiful bean seedlings at the end of each experiment (30-45 days).

Vector transmission of M-BCMV was studied in the greenhouse. Three aphid species, *Aphis craccivora* Koch, *Acyrthosiphon pisum* (Harris), and *Acyrthosiphon sesbaniae* David, were used. Nonviruliferous colonies of each aphid species were reared in cages on healthy broadbeans (*Vicia faba* L.). Aphids were starved 1-3 h in clean petri dishes before being given acquisition feeding periods of less than 1 min (duration of a single probe) to 24 h on M-BCMV-infected mungbean or bean.

Twenty virus-infected mungbeans from 13 lines in the observation trials at Karaj, Iran, were tagged before they flowered. Twenty healthy-appearing plants were tagged when they started to form pods. After harvest, part of the seeds from each line were planted in pasteurized soil in the greenhouse, where observations were made on seed transmission. The remaining seeds from disease-free and virus-infected plants from each line were planted in the field the following year. Plots were sprayed at frequent intervals with systemic insecticides. Seed yields from disease-free and virus-infected plants in each line were recorded at harvest time.

To determine whether M-BCMV was also seed-borne in *P. vulgaris,* Bountiful bean plants were inoculated in the seedling stage in the greenhouse. Seeds harvested from these plants were planted in pasteurized soil, where observations were made on seed transmission.

Drop-agglutination tests were performed by D. Z.

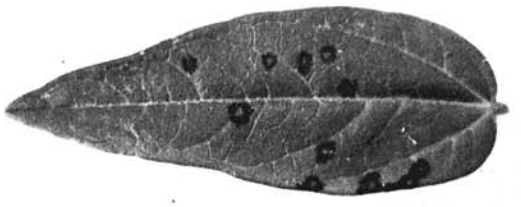

Fig. 1. Reddish-brown local lesions on primary leaf of Oklahoma 12 mungbean inoculated with the mungbean strain of bean common mosaic virus.

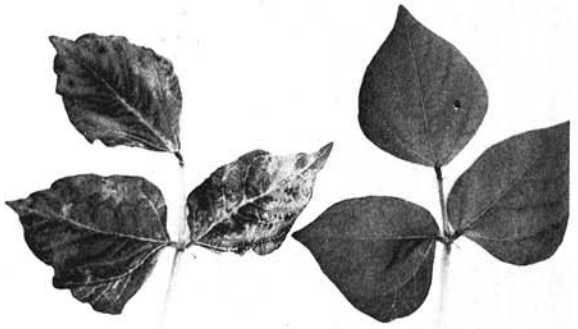

Fig. 2. Symptoms produced by the mungbean strain of bean common mosaic virus in mungbean. Trifoliate leaf from infected (left) and healthy (right) plant.

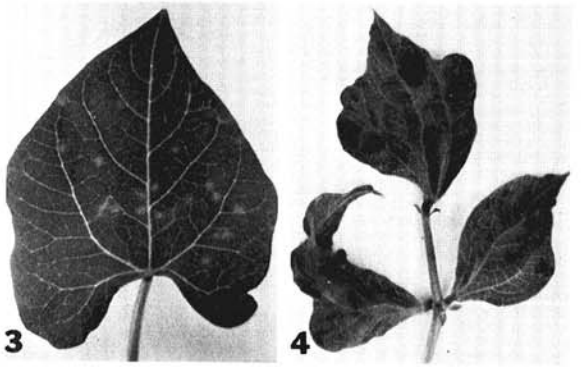

Fig. 3-4. 3) Chlorotic local lesions that developed on the primary leaf of Pinto U. I. 111 bean inoculated with the mungbean strain of bean common mosaic virus (M-BCMV). 4) Systemic symptoms of M-BCMV on Stringless Green Refugee bean.

Maat, Instituut voor Plantenziektundig Onderzoek, Wageningen, The Netherlands with sap from M-BCMV-infected bean and antiserum to a Dutch isolate of BCMV. Electron micrographs of negatively stained (2.0% potassium phosphotungstic acid, pH 6.6) leaf dip preparations of virus-infected bean and mungbean were prepared by F. Eskandari, Faculty of Agriculture, University of Tehran, Karaj, Iran, and the late J. Brandes, Biologische Bundesanstalt für Land-und Forstwirtschaft, Braunschweig, Germany.

In some experiments, an Iranian bean isolate of BCMV (B-BCMV) was included for comparison. The virus was isolated from bean seedlings infected from seed at Karaj.

Physical properties were tested according to the procedures of Bos et al. (2). Bountiful bean was used as a virus source plant and indicator host.

Test plants included in the host range or physical property studies, and plants used to maintain virus isolates or increase virus inoculum were grown in pasteurized greenhouse soil. All plants, unless otherwise specified, were sprayed at periodic intervals with one or more insecticides. Temperatures in the greenhouse ranged from 10-30 C.

Mungbean germplasm plantings were surveyed at periodic intervals during 1966 and 1969 at Karaj, where the incidence of M-BCMV was high. Plants from resistant and susceptible lines were also indexed on different indicator hosts to correlate the incidence of virus infection with the presence or absence of virus symptoms.

RESULTS.—*Host range studies.*—With the exception of *Chenopodium amaranticolor* Coste & Reyn. and *Nicotiana clevelandii* Gray, the host range of M-BCMV was limited to the Leguminosae (Table 1). M-BCMV produced systemic symptoms in bean, and mungbean, and several other legumes, including lentil (*Lens culinaris* Medic.), fenugreek (*Trigonella foenum-graecum* L.), and *Vicia narbonensis* L. Reddish-brown local lesions usually developed on primary leaves of mungbean 3-5 days after inoculation (Fig. 1). Subsequently, the virus went systemic, producing mosaic, deformation, puckering, rolling, and blistering symptoms on the foliage (Fig. 2) and stunting of the plant. Chlorotic or necrotic local lesions formed on the primary leaves of some bean lines 3-5 days after inoculation with M-BCMV (Fig. 3). Systemic symptoms of M-BCMV in susceptible bean varieties were similar to those produced by this virus in mungbean (Fig. 4).

M-BCMV and B-BCMV differed in host range and symptomatology (Table 1). Local lesions did not form on mungbean or tepary bean (*Phaseolus acutifolius* var. *latifolius* Freeman) inoculated with B-BCMV, nor did the virus produce necrotic local lesions on the bean cultivar Monroe. Both M-BCMV and B-BCMV were usually recovered from infected, but symptomless, hosts in back-inoculations to healthy Bountiful bean, which was highly susceptible to all Iranian mungbean and bean isolates of BCMV tested.

Seed transmission.—Seed transmisison of M-BCMV in 12 mungbean lines infected as seedlings, ranged from 8 to 32% (Table 2). Mosaic symptoms were often observed in the primary leaves of M-BCMV-infected seedlings and usually became more discernible in the trifoliate leaves that developed later. At times, virus symptoms did not appear until the second or third trifoliate leaf. M-BCMV was recovered consistently from seedlings showing mosaic and leaf deformation symptoms, but not from apparently healthy plants.

M-BCMV was also found to be seed-borne at a rate of 6.8% in Bountiful bean, when plants were infected before flowering (Table 3). In the same test, B-BCMV was transmitted in 34.8% of the Bountiful bean seed.

TABLE 1. Host range of two Iranian strains of bean common mosaic virus from bean (B-BCMV) and mungbean (M-BCMV)

Hosts	Virus isolates	
	B-BCMV	M-BCMV
Amaranthaceae		
Gomphrena globosa L.	–[a]	–
Chenopodiaceae		
Chenopodium amaranticolor Coste & Reyn.	CLL	CLL
Compositae		
Zinnia elegans Jacq.	–	–
Cucurbitaceae		
Cucumis sativus L. 'National Pickling'	–	–
Leguminosae		
Cassia obtusifolia L.	LL	LL
Cicer arietinum L. 'Ghazvin'	SI	SI
Glycine max (L.) Merr. 'Chippewa'	–	–
Lens culinaris Medic. 'Ghazvin'	–	M
Medicago sativa L.	–	–
Phaseolus acutifolius var. *latifolius* Freeman	M	LL,M
P. lunatus L. 'Jackson Wonder'	M	M
P. vulgaris L. 'Stringless Black Valentine'	–	–
P. vulgaris 'Bountiful'	CLL,M,LD,St	CLL,M,LD,St
P. vulgaris 'Columbia Pinto'	M	LL,M
P. vulgaris 'Great Northern U.I. 31'	SI	SI
P. vulgaris 'Great Northern U.I. 123'	M	M
P. vulgaris 'Great Northern U.I. 1140'	M	M
P. vulgaris 'Idaho Bountiful'	–	–
P. vulgaris 'Monroe'	VN	LL
P. vulgaris 'Pinto U.I. 111'	CLL,SI	CLL,M
P. vulgaris 'Pinto U.I. 114'	SI	M
P. vulgaris 'Red Mexican U.I. 34'	M	M,St
P. vulgaris 'Red Mexican U.I. 36'	M	M
P. vulgaris 'Resistant Asgrow Valentine'	–	–
P. vulgaris 'Sanilac'	M	M
P. vulgaris 'Stringless Green Refugee'	M,LD,St	M,LD,St
P. vulgaris 'Tendercrop'	–	SI
P. vulgaris 'Topcrop'	–	–
P. vulgaris 'U.S. 5 Refugee'	–	SI
P. vulgaris 'Wade'	–	–
Pisum sativum L. 'Rondo'	–	–
Trigonella foenum-graecum L.	M	M
Vicia faba L. 'Algerian'	–	–
V. narbonensis L.	SI	M
Vigna radiata (L.) Wilczek 'Berken'	SI	LL,M
V. radiata 'Oklahoma 12'	SI	LL,M
Vigna unguiculata (L.) Walp. 'Early Ramshorn'	–	SI
Solanaceae		
Capsicum annuum L. 'Anaheim'	–	–
Datura stramonium L.	–	–
Nicotiana clevelandii Gray	SI	M
N. glutinosa L.	–	–
N. tabacum L. 'Samsun'	–	–

[a]Hosts are listed alphabetically by family and genus.
[b]–, not susceptible; LL, local lesions; CLL, chlorotic local lesions; LD, leaf deformation; M, mosaic; SI, symptomless infection; St, stunting; VN, vein necrosis.

Effects of virus infection on yield.—M-BCMV adversely affected yields of virus-infected mungbean plants (Fig. 5). Dwarfing of plant growth was especially noticeable when infection occurred before pod-set. Seed yields from 11 mungbean lines grown under field conditions were decreased by 31-75% when infected from seed (Table 2). The seeds from M-BCMV-infected plants were often discolored, deformed, and smaller in size. At times, the germination of seeds from diseased plants was erratic and reduced. Growth and yields from susceptible bean varieties were also greatly decreased by M-BCMV (Fig. 6).

Physical property tests.—The in vitro properties of M-BCMV were as follows: Thermal inactivation, infection after heating for 10 min at 60 C, but none at 65 C; dilution end-point, infection at 10^{-3}, none at 10^{-4}; longevity in vitro at 20 C, infection at 14 days, but not at 17 days. The physical properties of B-BCMV were very similar to those of M-BCMV.

Serology and electron microscopy studies.—M-BCMV and B-BCMV were shown to be related serologically to a Dutch isolate of BCMV. In drop-agglutination tests, M-BCMV reacted with the Dutch BCMV antiserum at dilutions up to 1/256, which was close to the titer of the antiserum. Electron micrographs of negatively-stained leaf-dip preparations from mechanically-infected bean and mungbean revealed flexuous particles approximately 750 nm in length. Similar particles were also observed in leaf dip preparations of mungbean plants naturally infected with M-BCMV from seed or by aphids, indicating that infection was associated with flexuous, rod-shaped virus particles. No virus particles were seen in preparations from healthy plants.

Vector transmission studies.—Three aphid species, *Aphis craccivora, Acyrthosiphon pisum,* and *Acyrthosiphon sesbaniae,* were included in the transmission studies, because field observations and insect surveys implicated them as principal vectors of M-BCMV in Iran. The aphids acquired M-BCMV from virus-infected bean or mungbean in a stylet-borne (nonpersistent) manner with brief feeding probes of less than 2 min. Transmission was usually higher when Bountiful bean was used instead of mungbean both as a source and indicator plant. The disease symptoms resulting from aphid transmission of M-BCMV to mungbean in greenhouse tests were identical to those which developed on naturally infected plants in the field.

Varietal resistance.—In 1966, 1,112 mungbean accessions from 15 different countries were screened

TABLE 2. Effect of infection by the mungbean strain of bean common mosaic virus and subsequent seed transmission in mungbean lines planted in field trials at Karaj, Iran

Line (no.)	Country of origin	Decrease in yield (%)	Seed transmission (%)
1	USA	43[a]	22[b]
2	Iran	75	13
3	Iran	–	8
4	Iran	–	21
5	Iran	66	32
6	Iran	66	11
7	Iran	70	18
8	Pakistan	44	12
9	Iran	42	22
10	Iran	49	21
11	Iran	70	–
12	Iran	31	12
13	Iran	64	16

[a]Yields were taken from 20 diseased and 20 healthy plants in the same plot. – = no data collected.
[b]After harvest, seeds were planted in pasteurized soil in the greenhouse.

under natural field conditions at Karaj, Iran, against M-BCMV and other diseases. More than 18% of the lines were free of M-BCMV symptoms. In 1969, 663 of the lines included in the 1966 trials were again screened at Karaj for resistance to M-BCMV. The incidence and distribution of M-BCMV was higher in 1969 than in 1966. Many of the lines which exhibited resistance (disease rating 1) to M-BCMV in 1966 were highly susceptible (disease rating of 6-10) in the 1969 tests. Only two (0.3%) of the lines in the 1969 trials remained disease-free. M-BCMV was rarely isolated from healthy-appearing mungbean plants in the highly resistant lines.

TABLE 3. Transmission of two strains of bean common mosaic virus from bean (B-BCMV) and mungbean (M-BCMV) in seed of Bountiful bean[a]

Virus isolate	Seeds planted[b] (no.)	Seeds emerged (no.)	Seedlings infected from seed (no.)	Seed transmission (%)
B-BCMV	290	218	76	34.8
M-BCMV	241	206	14	6.8

[a]Bountiful bean plants were infected with each isolate of the virus in the greenhouse before plants had flowered.
[b]Values represent the average of two experiments.

DISCUSSION.—Several criteria were used to identify the mungbean virus as a strain of BCMV. These included symptomatology, host-range studies, physical property tests, vector and seed transmission, serology, and electron microscopy. These same criteria were also used to identify other isolates of BCMV in Iran, especially those affecting bean. Considerable variation in symptoms, host range, and physical properties exists among different identified strains of BCMV (1, 7, 8). Our results with M-BCMV were in general agreement with those of others for various strains of BCMV (1, 7, 8). Although it was not possible to compare isolates of M-BCMV under Iranian conditions

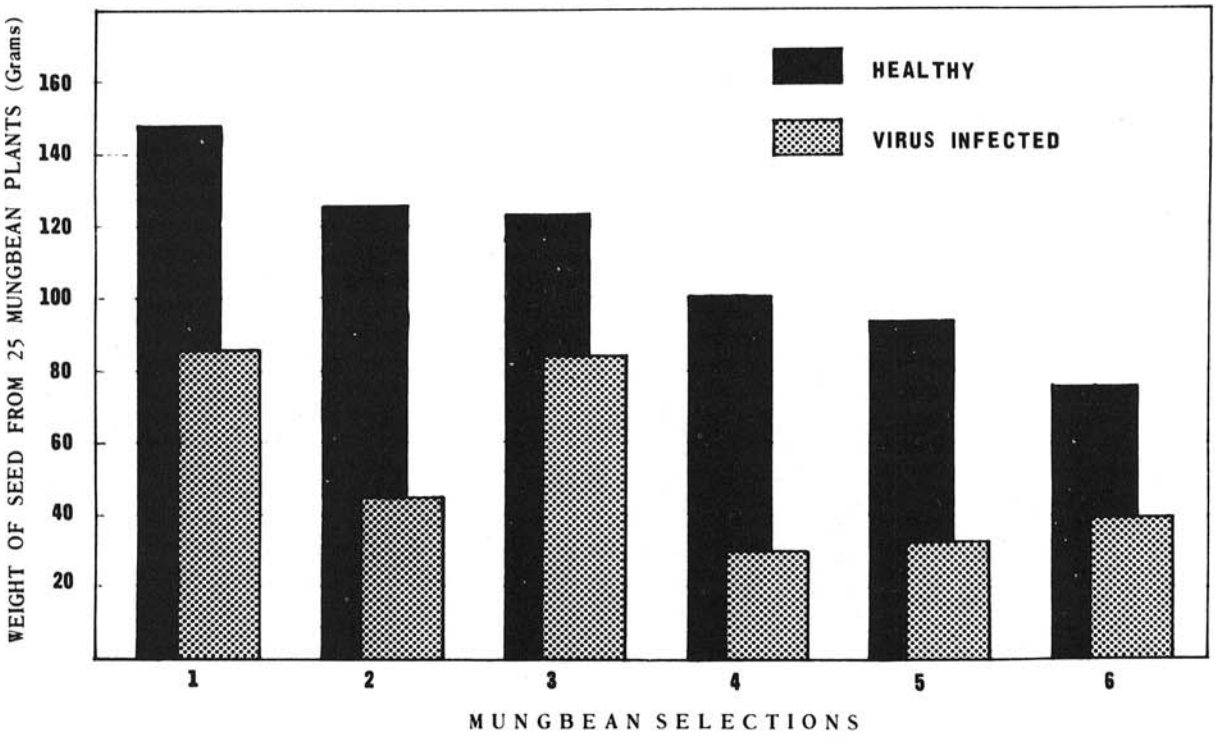

Fig. 5. Effects of the mungbean strain of bean common mosaic virus on seed yields of mungbean. Diseased plants were infected from seed.

Fig. 6. Effects of the mungbean strain of bean common mosaic virus on growth of Stringless Green Refugee bean (right). Healthy plants on left.

with known strains of BCMV from other countries because of quarantine regulations, it appears that several distinct strains of BCMV affect bean and mungbean in Iran.

In addition to M-BCMV, mungbeans in Iran are also affected by two other aphid-borne viruses, alfalfa mosaic (AMV) and cucumber mosaic (CMV) (4). The distribution of AMV and CMV is much more restricted than M-BCMV. AMV commonly infected mungbeans that were planted near alfalfa fields (*Medicago sativa* L.), the main forage crop and reservoir of the virus in Iran. Symptoms are useful in distinguishing M-BCMV from AMV, which produces yellow mosaic symptoms on the foliage of mungbean. However, the symptoms of M-BCMV and CMV are very similar and could be confused. Therefore, other procedures should be used to identify these viruses, such as host range studies, serology, and electron microscopy. AMV and CMV did not appear to be seed-borne in mungbean.

The mungbean germplasm nursery, consisting of 1,112 accessions from 15 countries, was first established at Karaj in 1966. Even though the incidence of M-BCMV was high, many lines that were thought to be resistant in the 1966 trial appeared to have escaped infection. In 1969, environmental factors seemed to be ideal for development and spread of M-BCMV at Karaj, thereby offering an excellent opportunity to locate sources of resistance to the virus in the mungbean germplasm. Only two lines out of 663 remained disease-free. In India, Nene (5) found two mungbean lines (Hybrid 45 and T-2) that were resistant to mosaic mottle. One of these lines, Hybrid 45, was also included in the 1969 Karaj trials, and it was one of the two lines that remained disease-free. This indicates that the factors governing resistance in mungbean to mosaic mottle and M-BCMV are similar, and that the two viruses are either closely related or identical.

The incidence and distribution of M-BCMV in commercial bean plantings in Iran are not known. It is very likely that beans are infected naturally by M-BCMV, especially in areas where virus-infected mungbeans are grown near susceptible bean fields. Many of the commercial bean varieties grown in Iran are susceptible to M-BCMV and other strains of BCMV. The spread of M-BCMV into areas where M-BCMV-susceptible bean varieties are cultivated could pose a serious threat to the bean industry of Iran, because of the adverse effects of virus infection on growth and yields of bean. In field and greenhouse inoculation trials with M-BCMV, bean seed yields were reduced by 40 to 100%, depending on the stage of plant growth at the time of infection (W. J. Kaiser, *unpublished*). Since M-BCMV is seed-borne in bean, the virus could be carried in bean seed to areas previously free of M-BCMV. Secondary spread of the virus would be accomplished by several aphid vectors that transmit M-BCMV in a stylet-borne manner.

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