BEAN COMMON MOSAIC VIRUS ISOLATED FROM MUNGBEAN (*VIGNA RADIATA*) IN THAILAND

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Abstract

A seed-borne, aphid-transmitted virus was found infecting mungbean (*Vigna radiata*) in various regions of Thailand. The pathogen was seed-borne in mungbean and bean (*Phaseolus vulgaris*). The plants showing systemic symptoms by mechanical inoculation of the virus were limited to the Leguminosae. Infectivity of crude sap of leaves was lost by heating at 50—60°C for 10 minutes, by diluting to 10^{-3}-10^{-4}, and by aging at room temperature for 4—8 days. The virus consisted of flexuous filaments about 750 nm in length and was transmitted by aphids in a non-persistent manner. In double-diffusion tests in agar gel plates containing 0.5% lithium 3,5-diiodosalicylate, the virus reacted strongly with antisera to bean common mosaic virus (BCMV) isolated from bean, blackeye cowpea mosaic virus, and azuki bean mosaic virus. Based on host range studies, symptoms, seed and vector transmission, serology, and particle morphology, the virus was identified as a strain of BCMV.

1. Introduction

Bean common mosaic virus (BCMV) isolated from mungbean (*Vigna radiata*) in Iran was described by Kaiser and Mossahebi (3). This virus causes the most important disease affecting mungbeans in Iran. In 1979, mungbean plants showing mosaic symptoms were observed in most areas where the crop was cultivated in Thailand. A virus was isolated from these plants and identified as a strain of BCMV. This paper reports the identification and characterization of the virus.

2. Materials and methods

1) Source of the virus isolated and antisera Two isolates of BCMV (M-8 and M-12) used in this study were recovered from naturally infected, field-grown mungbeans in Thailand in 1979. They were propagated on bean (*Phaseolus vulgaris* 'Honkintoki'). Some antisera were used to compare the serological relationships. The source of each antiserum was as follows: azuki bean mosaic virus (AzMV) (N. Iizuka, Hokkaido Natl. Agric. Exp. Stn. Sapporo); potato virus Y (Y. Saito, Natl. Inst. Agro-Envir. Sci. Tsukuba); beet mosaic virus (I. Fujisawa, Veget. and Ornam. Crops Res. Stn., Mie.); bean common mosaic virus, blackeye cowpea mosaic virus, soybean mosaic virus, bean yellow mosaic virus and turnip mosaic virus (T. Tsuchizaki).

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2) Mechanical inoculations Inoculations were performed by rubbing crude sap in 0.1 M phosphate buffer, pH 7.0, on leaves previously dusted with Carborundum. Infection or lack of infection of test cultivars and species was confirmed by lesion assays on *Chenopodium amaranticolor* or systemic assays on bean.

3) Insect transmission *Aphis craccivora* were used. Aphids were starved for 1—2 hr in clean petri dishes, then fed for 1—5 min leaf pieces from source plants. Groups of five aphids were transferred to individual plants of bean and 12 hr later the aphids were killed with insecticides.

4) Seed transmission To determine whether M-8 and M-12 were seed-borne in some species the plants were inoculated in the seedling stage in a greenhouse. Seeds harvested from these plants were planted in pasteurized soil, where observations were made on seed transmission.

5) Electron microscopy For electron microscopic examination of particle length of partially purified virus, preparations were mounted on a carbon-coated collodion grid and negatively strained in neutralized 1% phosphotungstic acid (PTA). Samples for electron microscopy also were prepared by the dip method directly from infected leaves into a drop of 1% PTA on a carbon-coated collodion grid.

6) Purification Infected tissue was homogenized in a mortar with 0.5 M citrate buffer, pH 7.2, containing 1% 2-mercaptoethanol. Homogenates were strained through double layers of cheesecloth and carbon tetrachloride was added to the extract to reach a final concentration of 20% (v/v). After stirring for 15 min, the emulsions were broken by centrifugation at 8,000 g for 15 min. Aqueous phases were treated by 5% of polyethylene glycol 6,000 (PEG) and 1% of Triton X-100 for 60 min, and then centrifuged at 8,000 g for 15 min. Resulting pellets were resuspended with 0.5 M phosphate buffer, pH 7.2, containing 0.01 M $MgCl_2$, and were centrifuged at 8,000 g for 10 min. After these PEG treatments were repeated twice resulting virus preparations were ultracentrifuged at 105,000 g for 90 min. Pellets were resuspended with the same buffer, and centrifuged at 6,000 g for 10 min. Sucrose density gradients were applied in columns of 10—40% sucrose in 0.5M phosphate buffer containing 0.01 M $MgCl_2$ at 60,000 g for 3 hr. Visible virus band was collected with an ISCO fraction collector scanning at 254 nm, and centrifuged at 120,000 g for 90 min. The virus pellets were resuspended in 0.005 M phosphate buffer, pH 7.0.

7) Serology Antiserum against M-12 was prepared in a rabbit given three intramuscular injections and then one intravenous injection of purified virus. Ouchterlony double-diffusion tests were conducted in 0.8% agar with 0.85% sodium chloride, 0.5% lithium 3,5-diiodosalicylate, and 0.1% sodium azide.

3. Results

1) Host range studies

With the exception of five species ie *Chenopodium,* spp. *Tetragonia expansa* and *Sesamum indicum,* the host range of M-8 and M-12 was limited to the Leguminosae (Table 1). Both isolates produced systemic symptoms in bean, azuki bean, blackeye

Table 1. Reactions of selected plant species and cultivars to M-8 and M-12 isolates of bean common mosaic virus.

Genus, species, cultivar	Reaction			
	M-8		M-12	
	Inoculated leaves	Noninoculated portions of plant	Inoculated leaves	Noninoculated portions of plant
Legumes				
Glycine max 'Isuzu'	CS, NRS[a)]	—	CS, RRS	—
'Shirotsurunoko'	CS, NRS	—	CS, NRS	—
'Tokachinagasaya'	VN	—	VN	CS, VN
Pisum sativum 'Kinusaya'	—	—	—	—
Phaseolus angularis 'Wasedairyu'	LL	M	LL	M
P. vulgaris 'Kairyootebo'	NRS, VN	CS, M, NS	CS, MRS	M, NS
'Honkintoki'	CS, VN	M	CS, NS	M
'Top Crop'	—	—	—	—
'Wasedaifuku'	NS	Mt, RL	NS, VN	Mt, MR
Trifolium pratense	—	—	—	—
T. repens	—	—	—	—
Vicia faba	LL	LS	LL	LS
Vigna mungo	LL	M, RL	LL	M
V. radiata 'M7A'	LL	VC, M	LL	VC, M
V. sesquipedalis 'Kurodanesanjaku'	—	—	—	—
V. sinensis 'Blackeye Cowpea'	CS	M	CS	M
Nonlegumes				
Capsicum annuum	—	—	—	—
Chenopodium amaranticolor	—	—	CS	—
C. album	—	—	CS	—
C. quinoa	LL	—	CS	—
Nicotiana glutinosa	—	—	—	—
N. tabacum 'Samsun'	—	—	—	—
Petunia hybrida	—	—	—	—
Sesamum indicum	LL	—	LL	—
Tetragonia expansa	—	—	LL	—
Zinia elegans	—	—	—	—

a) NRS: necrotic ringspot, VN: vein necrosis, CS: chlorotic spot, M: mosaic, NS: necrotic spot, Mt: mottle, RL: rugose leaf, LL: symptomless local infection, VB: vein banding, LS: symptomless systemic infection, —: no infection.

cowpea, *Vigna mungo* and mungbean. M-8 and M-12 differed slightly in host range and symptomatology.

2) Seed transmission

Seed transmission of M-8 and M-12 in several species was tested. M-8 and M-12 were transmitted in 4.1% (7/172) and 7.2% (18/248) of mungbean (cv. 'M 7 A') seed respectively. M-12 was also found to be seed-borne at a rate of 1.3% (1/77) in bean (cv. 'Honkintoki').

3) Insect transmission

M-8 and M-12 were transmitted from bean to bean by *Myzus persicae* in a non-

persistent manner. M-12 was also transmitted by *Aphis craccivora*. *Aulacorthum solani* was able to transmit M-8 but not M-12.

4) Properties in vitro

The in vitro properties of M-12 were as follows. The thermal inactivation point (10 min) ranged between 50 and 60°C; Dilution end point ranged between 1:1,000 and 1:10,000; longevity in vitro at room temperature ranged between 4 and 8 days.

5) Electron microscopy

Electron micrographs of negatively-stained leaf-dip preparations from bean plants mechanically infected with M-8 and M-12 respectively revealed the presence of flexuous particles approximately 750 nm in length. Similar particles were also observed in purified preparations of M-12.

6) Serology

In double-diffusion tests with agar containing 0.5% lithium 3,5-diiodosalicylate the antigenic relatedness of M-8 and M-12, BCMV, BlCMV and AzMV was serologically identical (Table 2). Antisera against SMV-H and SMV-27 also reacted with M-8 and M-12, forming a distinct spur. M-8 and M-12 did not react with antiserum to several other potyviruses including BYMV, PVY, TuMV and BMV.

Table 2. Serological reactions of M-8 and M-12 isolates of bean common mosaic virus to antisera against several viruses belonging to potyviruses in agar gel diffusion plates containing 0.5% lithium 3,5-diiodosalicylate

Antisera	Antigens	
	M-8	M-12
Bean common mosaic virus isolate from mungbean (M-12)	+	+
Bean common mosaic virus isolate from bean	+	+
Blackeye cowpea mosaic virus		+
Azuki bean mosaic virus	+	+
Soybean mosaic virus from Japan	+*	+*
Soybean mosaic virus from Thailand	+*	+*
Bean yellow mosaic virus	-	-
Potato virus Y	-	-
Turnip mosaic virus	-	-
Beet mosaic virus	-	-

* Spur reaction was observed with the virus isolate homologous to the antiserum used.

4. Discussion

The virus recovered from mungbean in Thailand was identified as BCMV on the basis of particle size and morphology, physical properties, host range and symptomatology, transmission and serology. In 1968, a seed-transmitted virus disease of mungbean was described in Iran by Kaiser *et al* (2). The virus disease was called mungbean mosaic virus and appeared to be related to BCMV. In 1974, the mungbean virus was identified as a strain of BCMV (M-BCMV) (3). The results of our findings on M-8 and M-12 were in general agreement with those on M-BCMV.

M-8 and M-12 differed in their infectivity to *C. amaranticolor* and *C. album,* and in transmission by aphid. Some variations in the symptoms and host range were observed among different identified strains of BCMV (1). It appears that several distinct strains of BCMV affect mungbean in Thailand.

Since BCMV isolated from mungbean in Thailand was seed-borne in mungbean and bean, the virus could be carried in seeds of mungbean and bean to areas previously free of BCMV. Secondary spread of virus could be achieved by several aphid vectors that transmit BCMV in a stylet-borne manner.

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