Identification and Partial Characterization of a Strain of Bean Common Mosaic Virus from Rhynchosia minima

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We thank C. W. Rogers, D. K. Froh, and S. S. Hearon for technical assistance and R. Provvidenti for BCMV and BYMV antisera.

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Accepted for publication 30 August 1977.

ABSTRACT

MEINERS, J. P., A. G. GILLASPIE, JR., R. H. LAWSON, and F. F. SMITH. 1978. Identification and partial characterization of a strain of bean common mosaic virus from Rhynchosia minima. Phytopathology 68: 283-287.

A virus was mechanically transmitted to bean (*Phaseolus vulgaris*) from *Rhynchosia minima* plants growing adjacent to bean plantings in Colombia. The virus produced mosaic, stunting, and leaf-curling in *R. minima* and symptoms in Stringless Green Refugee bean identical to those caused by bean common mosaic virus (BCMV). Based on differential host reactions, the virus is a member of BCMV strain group I. The virus is seed-transmitted in bean but not in *R. minima*, and is aphid-transmitted in both hosts. Virion morphology and physical properties are similar to BCMV and the virus is serologically related to BCMV. This is the first report of BCMV occurring naturally in a noncultivated host. *Rhynchosia minima* infected with BCMV could serve as a source of virus for infection of cultivated beans because it is a common weed adjacent to bean fields in tropical areas.

In November 1974 a *Rhynchosia minima* (L.) DC. plant showing mosaic symptoms was found growing adjacent to an experimental bean planting at the Centro Internacional de Agricultura Tropical (CIAT), Palmira, Colombia. The plant was taken to the Beltsville Agricultural Research Center where a virus was isolated from it and identified as a strain of bean common mosaic virus (BCMV). This paper reports the identification and characterization of the virus that we will refer to as BCMV-R. A preliminary report has been published (4).

MATERIALS AND METHODS

Virus source.—The virus was mechanically transmitted from *Rhynchosia minima* to Stringless Green Refugee (SGR) bean and was maintained in plants or seed of that cultivar. Infected seed of SGR was used as a source of inoculum for studies of host range, insect transmission, in vitro properties, purification, serology, and electron microscopy. The studies were repeated with virus obtained from three serial local lesion transfers on Monroe bean and *Dolichos lablab* L. and maintained in plants or seed of SGR.

Mechanical inoculations.—Inoculations were made by rubbing crude sap or purified virus in 0.01 M potassium phosphate buffer, pH 7.0, on primary leaves previously dusted with 45-μm (320-mesh) carborundum. Infection or lack of infection on test cultivars and species was confirmed by lesion assays on Monroe bean cultivar and/or *Dolichos lablab* and systemic assays on SGR. All inoculations were accompanied by noninoculated control plants. The reaction of BCMV-R to differential bean cultivars was compared with that of BCMV type strain (ATCC No. PV 25) (6) by use of the same bean cultivar as the source of virus, inoculation of similar test plants with equivalent amounts of virus, and incubation of plants under identical conditions.

Insect transmission.—Apterous green peach aphids, *Myzus persicae* (Sulz.), used in transmission tests were from a laboratory colony reared on seedling *Datura stramonium* L. in a growth chamber (20 C and a 16-hr photoperiod). Test aphids were brushed from host leaves into Stender dishes, starved for 2 hr, then fed for 10 min on leaf pieces from source plants. Aphids, on leaf pieces, were placed, 15 per plant, on small pieces of toweling positioned on test plants so that aphids could move at will to test plants. After a 3-hr inoculation feeding, the source leaf pieces and toweling were removed, the test plants were dipped in water containing nicotine sulfate to kill aphids present, and the plants were grown for infection readings in an insect-free greenhouse.

Virus purification.—The virus was partially purified by modification of the method described by Huttinga (3) for bean yellow mosaic virus (BYMV). Systemically infected leaves of SGR, harvested 13 days after inoculation, were extracted in 1.5 volumes of 0.1 M tris-thioglycolic acid, pH 9, with 0.4 volumes each of chloroform and carbon tetrachloride in a blender. The resulting emulsion was broken after 10 min at 7,500 *g*. Then the aqueous phase was centrifuged at 23,000 *g* for 1.5 hr and the pellets were resuspended in 0.1 M tris-HCl, pH 9. Resuspended pellets

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were clarified at 4,600 *g* for 10 min. The supernatant was centrifuged through a 10-40% linear sucrose gradient in 0.1 M tris-HCl, pH 9, at 81,000 *g* for 1.75 hr. The virus zone was collected with a hypodermic needle and syringe and concentrated by centrifugation at 80,000 *g* for 2 hr or by ultrafiltration with an Amicon XM100A filter in a Model 52 filtration unit (Amicon Corp., Lexington, MA 02173). Final virus preparations were suspended in 0.02 M tris-HCl, pH 9.

Serology.—Tests were made with BCMV-R against antisera to BCMV and bean yellow mosaic virus (BYMV) in microprecipitin tests. Partially purified virus samples (0.5 mg/ml to 0.03 mg/ml) and antisera were diluted in 0.02 M tris-HCl, pH 9. The plates were incubated at room temperature and were read at 2 and 4 hr and again after being kept overnight at 4 C.

Electron microscopy.—Leaf-dip preparations were made, from SGR 13 days after inoculation, in 2% potassium phosphotungstate (PTA), pH 7.0, or in 3% neutralized ammonium molybdate. Length of virus particles was determined by comparison with 500 nm polystyrene latex beads photographed at the same magnification.

Partially purified BCMV-R was mixed with antisera to BCMV or to BYMV on the surface of a Formvar-coated grid and incubated for 5 hr. Then most of the droplet was removed and the virus-antiserum mixture was stained with potassium phosphotungstate. The virus was similarly incubated with normal serum and stained.

RESULTS

Mechanical transmission and symptoms.—No symptoms developed on Topcrop bean inoculated with crude sap from naturally infected *R. minima* when incubated either in the greenhouse (22-27 C) or growth chamber (30-32 C) and illuminated at 16,140 lx (1,500 ft-c) for 12 hr. No symptoms developed on inoculated SGR in the greenhouse, but symptoms typical of BCMV developed in the growth chamber.

TABLE 1. Symptom expression in host plants inoculated with the *Rhynchosia* strain of bean common mosaic virus and grown under controlled light and temperature conditions and in the greenhouse

	Greenhouse (21-30 C)		Growth chamber (32 C)	
Species inoculated	LL	Systemic	LL	Systemic
Phaseolus vulgaris:				
Stringless Green Refugee	0/6	6/6 M[a]	0/6	6/6 M
Topcrop	0/6	0/6	0/6	0/6
Monroe	6/6 N	0/6	0/6	0/6
ICA-Tui	0/6	1/6 N	0/6	3/6 N
Rhynchosia minima	0/6	6/6 M	0/6	5/6 M

[a]The indicated fractions represent number of plants with visible symptoms (numerator) among the total number that were inoculated.

[b]Abbreviations defined: LL = local lesions; M = mosaic; N = necrosis.

In a second experiment (Table 1) no symptoms developed on Topcrop inoculated with crude sap from infected SGR, but symptoms typical of BCMV developed on SGR in both greenhouse (21-30 C) and growth chamber (32 C) (Fig. 1-A).

Inoculated Monroe showed local symptoms typical of BCMV (8) and ICA-Tui showed a hypersensitive reaction typical of certain cultivars resistant to BCMV (Table 1). Symptoms developed on inoculated plants of *R. minima* incubated both in the growth chamber and greenhouse (Fig. 1-B). Mosaic and curling of the leaves and stunting of the plants were more severe in the growth chamber than in the greenhouse. Stringless Green Refugee plants inoculated with crude sap from infected *R. minima* produced typical BCMV symptoms in the growth chamber at 32 C, but remained symptomless in the greenhouse.

Seed transmission.—Symptoms of BCMV were observed in 33.5% (44/130) of the SGR seedlings grown

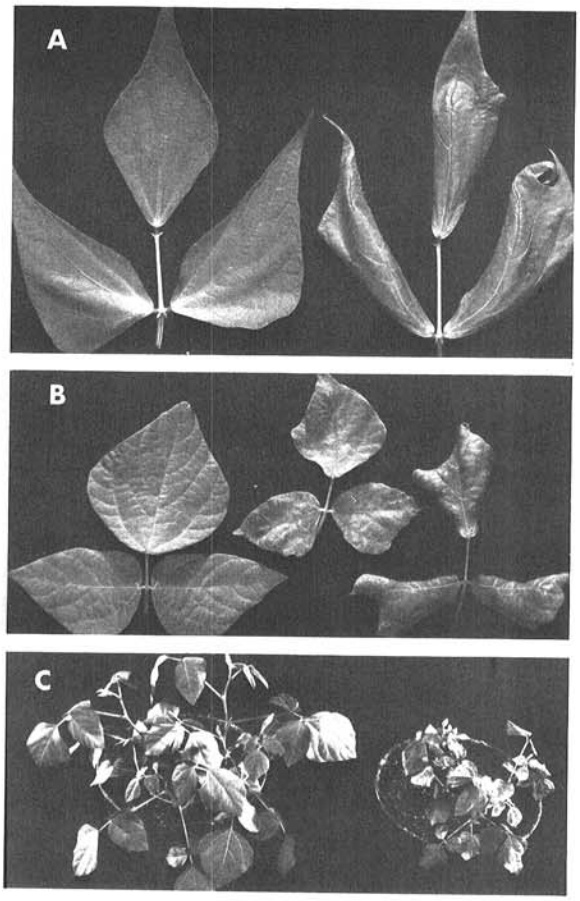

Fig. 1-(A to C). A) Leaves of Stringless Green Refugee bean that were (left) noninoculated or (right) inoculated with the *Rhynchosia* strain of bean common mosaic virus (BCMV-R). **B)** Leaves of *Rhynchosia minima* that were (left) noninoculated or (center and right) inoculated with BCMV-R. **C)** Plants of *Rhynchosia minima* (left) noninfected or (right) infected with BCMV-R through aphid (*Myzus persicae*) feeding.

from infected plants. No symptoms were observed in seedlings grown from infected seed of *R. minima* (0/113) or Henderson Bush lima bean (0/21), and the virus was not recovered on SGR or Monroe.

Insect transmission.—The virus was transmitted from SGR by aphids (*M. persicae*) to SGR (22 infected plants per 24 plants exposed) and to *R. minima* (22/33). By use of these plants as a source of virus, aphids subsequently transmitted the virus from *R. minima* to *R. minima* (9/24), from *R. minima* to SGR (5/18), SGR to SGR (7/12), and SGR to *R. minima* (3/8). Infection was confirmed by bioassay on SGR and Monroe. Symptoms on *R. minima* (Fig. 1-C) were the same as those produced by mechanical inoculation.

Properties in vitro.—In crude sap extracted from SGR and bioassayed on Monroe, BCMV-R was infectious after 10 min at 56 C but not at 59 C. It also was infectious after a dilution of 10^{-2} but not of 10^{-3}. In the same test, BCMV-type was inactivated at 62-65 C and had a dilution end point between 10^{-3} and 10^{-4}. The reported thermal inactivation point for BCMV is 50-65 C and the dilution end point is between 10^{-3} and 10^{-4} (1).

Purification.—After partial purification, the virus samples contained some green material and many of the particles were broken. As evidenced by reduced numbers of local lesions on Monroe, BCMV-R was recovered in lower concentrations than was BCMV-type strain from equivalent quantities of infected SGR plants grown under identical environmental conditions in the greenhouse.

Serology.—The BCMV and BYMV antisera had homologous titers of 4,096 and 2,048, respectively, in microprecipitin tests of Uyemoto et al. (9). Partially purified BCMV-R gave a positive test with BCMV antiserum at a dilution of 1/32. No specific reaction occurred with BYMV antiserum, but a nonspecific reaction occurred at the 1/2 and 1/4 dilutions.

Electron microscopy.—No reaction was observed between particles of BCMV-R and BYMV antiserum in negatively stained preparations in the electron microscope. Virions incubated with BCMV antiserum showed a thickened and diffused appearance along the surface of the virions indicating specific antibody attachment (Fig. 2-A). Virions incubated with normal serum were normal width when stained with PTA and there was no evidence of globulin attachment to the surface of the virions (Fig. 2-B).

Seventy-one percent of 118 particles measured in leaf-dip preparations ranged from 690-750 nm. No difference in length distribution was measured when virus particles stained with potassium phosphotungstate were compared to those stained with ammonium molybdate.

Strain relationships.—Differential cultivars reacted similarly to both BCMV-R and type (ATCC PV 25) BCMV. Only cultivars in Host Group 1 (Dubbele Witte, Sutter Pink, SGR) were infected systemically, which places both strains in Virus Strain Group I (2). When inoculated with equivalent amounts of virus, systemic symptoms produced on the cultivars by BCMV-R were milder than those of the type strain. Furthermore, BCMV-R produced only a few necrotic local lesions on Monroe and Red Mexican U.I. 35, whereas numerous lesions were produced by BCMV-type strain.

Seedlings of *R. minima* were inoculated with the type and New York (NY) 15 (ATCC PV 28) (7) strains of BCMV to determine susceptibility to strains other than BCMV-R. *Rhynchosia minima* also was inoculated with BCMV-R. The same source cultivar (SGR) and equivalent amounts of virus were used. *Rhynchosia minima* plants were incubated in the greenhouse (22-27 C) and growth room (32 C). Plants infected with type or NY 15 strains were severely stunted and necrotic in both the greenhouse and growth chambers. One mo after inoculation seven of 12 plants infected with the type, and nine of 10 of those infected with NY 15 were dead. All plants inoculated with BCMV-R showed only mild leaf curling and stunting and none were dead 1 mo after inoculation. Later, the test was repeated twice in the greenhouse with similar results.

Host range.—Of 25 species of legumes inoculated mechanically with BCMV-R, 13 (including *Phaseolus* vulgaris and *R. minima*) developed local or systemic symptoms or latent infection (Table 2). Some cultivars of *P. lunatus*, *Vigna radiata*, and *V. unguiculata* were immune. *Dolichos lablab* proved to be an excellent local lesion host, and was used both for single-lesion isolations and for indexing of inoculated plants in subsequent studies. Of seven nonleguminous species, only *Nicotiana clevelandii* proved to be susceptible (Table 2). This rather narrow host range is typical of many strains of BCMV.

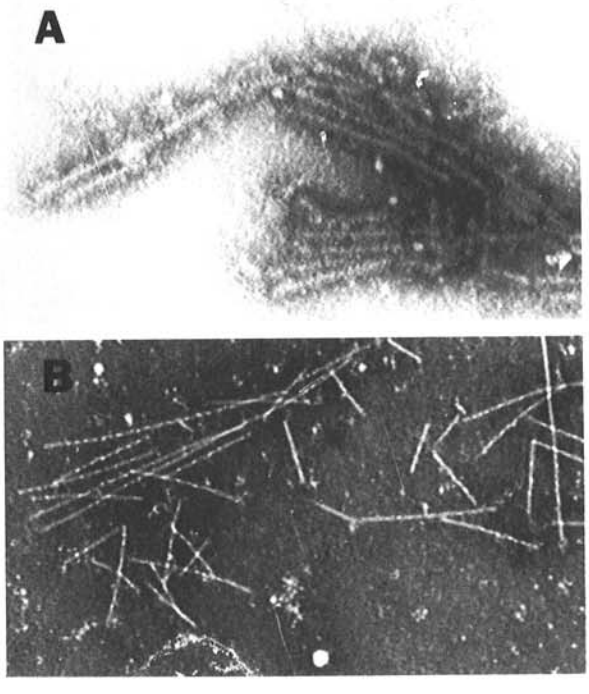

Fig. 2-(A, B). A) Virions of the *Rhynchosia* strain of bean common mosaic virus (BCMV-R) incubated with BCMV antiserum showing thickened and diffuse appearance on surface of the virions indicating specific antibody attachment. **B)** Virions of BCMV-R incubated with normal serum and stained with phosphotungstic acid, showing no antibody attachment.

DISCUSSION

The potyvirus recovered from *R. minima* was identified as bean common mosaic virus by serology, in vitro properties, and characteristic symptoms on bean cultivars. The virus is designated BCMV-R and is placed in Virus Strain Group I (2) on the basis of reaction to the BCMV differential bean cultivars.

Both the BCMV-R and BCMV-type strains produce similar infection patterns on specific BCMV differential bean cultivars, and from this standpoint, BCMV-R could be considered to be an isolate of the type strain. However, they differ in another important characteristic; namely, titer. The *Rhynchosia* strain appears to have a much lower titer in susceptible bean cultivars and in *R. minima* than does the type strain, as indicated by several comparative tests. The virus from *Rhynchosia* produced much milder symptoms on the differential cultivars and on *R. minima* than did the type and NY 15 strains. Similarly, it produced very few local lesions on Monroe, Red Mexican U.I. 35, and *Dolichos lablab*, whereas the type strain produced many local lesions. Finally, BCMV-R produced consistently lower yields of purified virus than did the type. However, the percentage of seed and insect transmission is similar to those of the type and other strains of BCMV as reported in the literature.

This is the first report of BCMV occurring on *R. minima*, although another member of this genus (*R. phaseoloides*) has been shown by inoculation to be susceptible to infection by BCMV (5). This, also, is the first report of BCMV occurring naturally on a noncultivated host.

It is well established that epidemics of common bean mosaic disease originate from seed-borne virus and spread by natural populations of aphids. In tropical areas where *R. minima* is a very common weed adjacent to or in bean fields, it may also serve as a source of epidemics of this disease. *Rhynchosia minima* is long-lived in field borders, and this would enhance its importance as a source of virus inoculum. This may complicate attempts to control the disease through the well recognized practice of providing virus-free seed for planting and emphasizes the desirability of control of BCMV through breeding for resistance. The extent to which natural populations of *R. minima* are infected with BCMV is unknown.

TABLE 2. Reaction of selected plant species and cultivars to the *Rhynchosia* isolate of bean common mosaic virus in crude sap

Genus, species, cultivar[a]	Reaction[b]	
	Inoculated leaves	Noninoculated portions of plant
Legumes:		
Dolichos lablab L.	LLN	TN
Macroptilium atropurpureum (DC.) Urb. 'Siratro'	None	Lat.
M. lathyroides (L.) Urb.	LLN	M, VN
Phaseolus acutifolius Gray 'Red Tepary'	None	M, LC, LLN
P. lunatus L. 'Henderson Bush'	LLN, VN	None
P. vulgaris L. Susceptible cultivars	LLN, LLC, None	M, LC
Rhynchosia minima (L.) DC.	None	M, LC, S
Sesbania exaltata (Raf.) Rydb.	None	Lat.
Vigna aconitifolia (Jacq.) Marechal	None	M, LC, LLN
V. angularis (Willd.) Ohwi & Ohashi	None	Lat.
V. mungo (L.) Hepper	LLN	LLN, S
V. radiata (L.) Wilczek 'Thailen'	None	LLN, LC, VB
V. unguiculata (L.) Walp. 'California Blackeye No. 5'	None	M
Nonlegumes:		
Nicotiana clevelandii Gray	None	Lat.

[a]Inoculation of the following species and cultivars resulted in no detectable infection: **Legumes**–*Arachis hypogaea* L. 'Tifspan', 'Florunner'; *Canavalia ensiformis* (L.) DC.; *Cassia obtusifolia* L.; *Cicer arietinum* L. 'No. 74', 'WCH No. 1'; *Crotalaria juncea* L.; *Cyamopsis tetragonoloba* (L.) Taub.; *Glycine max* (L.) Merr. 'Scott', 'Kanrich'; *Lathyrus sativus* L.; *Lens culinaris* Medic.; *Phaseolus coccineus* L. 'Kelvedon Marvel', 'Scarlet Runner'; *P. lunatus* L. 'Fordhook 242', 'Jackson Wonder'; *Pisum sativum* L. 'Alaska'; *Vicia faba* L. 'Bell Bean'; *Vigna radiata* (L.) Wilczek 'Berken', 'Moren'; *V. unguiculata* (L.) Walp. 'Yardlong'. **Nonlegumes**–*Chenopodium amaranticolor* Coste & Reyn.; *C. quinoa* Willd.; *Datura stramonium* L.; *Gomphrena globosa* L.; *Nicotiana glutinosa* L.; *Spinacea oleracea* L. 'Bloomsdale Long Standing'.

[b]Abbreviations defined: LLN = necrotic local lesions; TN = top necrosis; Lat. = latent systemic infection; M = mosaic; VN = vein necrosis; LC = leaf curling; LLC = chlorotic local lesions; S = stunting; VB = vein banding.

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