Properties of Blackgram Mottle Virus

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ABSTRACT

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Blackgram mottle virus was purified from infected Black Valentine bean plants by a chloroform-butanol procedure. Purified preparations contained polyhedral particles 28 nm in diameter and exhibited a single immunoelectrophoretic component that migrated toward the anode at pH 8.8. Analytical ultracentrifugation showed a single component with a sedimentation coefficient of 122S and a density of 1.364 gm/cm^3. Polyacrylamide gel electrophoresis demonstrated one nucleic acid component and one coat protein subunit with estimated molecular weights of 1.4×10^6 and 38,200 daltons, respectively. Phenol-extracted nucleic acid was infectious on Pinto bean and sensitive to ribonuclease and had a nucleotide ratio of G = 24.9%; A = 25.9%; C = 25.3%; U = 24.0%. Blackgram mottle virus was transmitted by the bean leaf beetle, *Cerotoma trifurcata*, and the Mexican bean beetle, *Epilachna varivestis*. Reciprocal serologic tests failed to show relationships between blackgram mottle virus and members of other beetle-transmitted legume virus groups.

Additional key words: Beetle-transmitted viruses.

In 1968, virus-infected blackgram, *Vigna mungo* L., was found near new Delhi, India (12). The virus was seedborne in about 5% of infected blackgram and had a 5×10^{-4} to 1×10^{-5} dilution end point, a thermal inactivation point of 90–92 C, and a longevity in vitro of 40–45 days. The virus was not transmitted by aphids and was limited to legumes. Electron microscopic examination of leaf-dip preparations showed isometric viruslike particles. Preliminary serologic tests indicated a relationship between the blackgram virus and broadbean mottle virus (12).

This article describes additional properties of blackgram mottle virus (BGMV) and shows it to be unrelated to broadbean mottle and other beetle-transmitted legume viruses. A preliminary report was published (14).

MATERIALS AND METHODS

Host range. Blackgram mottle virus was maintained in *Phaseolus vulgaris* L. 'Black Valentine.' Host range and symptomatology were determined by Phatak (12). A limited host range study was repeated, however, primarily to find a suitable local lesion host. The plants tested were bean, *P. vulgaris* 'Pinto' and 'Black Valentine'; mung bean, *P. aureus* L.; guar, *Cyamopsis tetragonoloba* (L). Taub; soybean, *Glycine max* L. 'Lee'; *Vicia faba* L.; and cowpea, *Vigna unguiculata* Walp. 'Monarch' and 'Crimson.' Cotyledons or primary leaves of 20–30 Carborundum-dusted plants of each species were inoculated with sap from infected Black Valentine bean. All plants not showing symptoms were assayed for virus on Black Valentine bean. The host range tests were repeated several times.

Purification. The virus was purified by extracting sap from Black Valentine bean 10–14 days after inoculation with 1.0–1.5 ml of 0.2 M phosphate buffer, pH 7.2, containing 0.1 M ascorbic acid and 1 ml of chloroform-butanol per gram of tissue. The extract was held overnight at room temperature, centrifuged at 5,000 *g* for 10 min, and then the aqueous phase was subjected to three alternate high (80,000 *g* for 1 hr) and low (9,000 *g* for 10 min) speed centrifugations. High speed pellets were resuspended in 0.01 M sodium phosphate buffer, pH 7.2.

Analytical ultracentrifugation. Two milligrams per milliliter of purified BGMV was centrifuged at 32,000 rpm in the An-D rotor of the Beckman analytical ultracentrifuge with Schlieren optics. Sedimentation coefficients were determined using Markham's graphic method (10).

Buoyant density centrifugations were made by mixing purified virus ($A_{260nm} = 0.04$) with CsCl in 0.01 M phosphate buffer, pH 7.2, and centrifuging for 24 hr at 44,000 rpm in the An-D rotor with ultraviolet optics. Density of the virus was determined by Chervenka's method (1).

Serology. Rabbits were given several weekly subcutaneous injections of purified BGMV plus Freund's incomplete adjuvant to a total of 7 mg of virus. The virus and its antiserum were tested in reciprocal gel diffusion tests against beetle-transmitted legume viruses, including broadbean mottle, southern bean mosaic, cowpea strain of southern bean mosaic, cowpea mosaic (Arkansas and Sb), bean pod mottle, cowpea chlorotic mottle, quail pea mosaic viruses (3), and two new beetle-transmitted viruses, bean mild mosaic (17) and cowpea mottle (16). Other reciprocal serologic tests included tobacco ringspot and Desmodium yellow mottle viruses.

Immunoelectrophoresis was performed with purified virus and Gelman high resolution buffer, pH 8.8 (Gelman Instrument Co., Ann Arbor, MI) as described by Scott and Moore (13).

Nucleic acid. Nucleic acid was extracted from BGMV by a phenol-sodium dodecyl sulfate (SDS) method (2). The base ratio was determined by the method described by Kaper et al (6). Sensitivity of the nucleic acid to ribonuclease was tested by mixing 1×10^{-3} μg/ml of bovine pancreatic ribonuclease (Sigma Chemical Co., St. Louis, MO 63178) with 1 μg/ml of nucleic acid. After incubation at 0 C for 30 min, Pinto bean was inoculated with the mixture. Half-leaf controls consisted of BGMV plus ribonuclease.

The molecular weight of the nucleic acid was estimated by polyacrylamide gel electrophoresis (7). Purified virus was mixed with Lane's (7) dissociation buffer immediately before electrophoresis in 2.9% gels. The standards were nucleic acids from southern bean mosaic (2), tobacco mosaic (4), and brome mosaic viruses (8).

Protein. The molecular weight of the BGMV protein subunit was determined by electrophoresis on 7.5% acrylamide gels (9). Purified virus was dissociated by mixing with 1% SDS and 1% dithiothreitol and heating at 100 C for 5 min. Markers for determination of molecular weight were bovine serum albumin, ovalbumin, pepsin, trypsin, chymotrypsin, hemoglobin (Sigma Chemical Co., St. Louis, MO), tobacco mosaic virus (4), and southern bean mosaic virus (5) proteins.

Dry weight and optical density relationship. Purified BGMV was dialyzed against distilled water and the A_{260nm} was read in a

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Beckman DB spectrophotometer. Samples of the material were dried to constant weight.

Electron microscopy. Purified BGMV was mixed with an equal volume of 2% phosphotungstic acid, pH 6.8, and applied to a parlodion-coated grid for examination in the electron microscope.

Beetle transmission. Bean leaf beetles, *Cerotoma trifurcata* (Forst.), and Mexican bean beetles, *Epilachna varivestis* (Muls.), were given acquisition feedings of 24 hr on infected Black Valentine bean. Single, caged beetles were then allowed to feed on healthy Black Valentine beans to test for transmission. The beetles were transferred daily for 4 days. Extracts from test plants were assayed for virus on Ouchterlony gel diffusion plates (11) after 3 wk.

RESULTS

Host range and symptoms. Inoculated primary leaves of Pinto bean, which was selected as the local lesion host, exhibited small, necrotic local lesions (Fig. 1A) within 3 days after inoculation, but systemic infection did not develop. Black Valentine bean, the virus source for purification, showed chlorotic spots on inoculated primary leaves and mosaic and distortion in trifoliolate leaves (Fig. 1B). Necrotic local lesions developed on inoculated guar cotyledons, but no systemic infection followed (Fig. 1C). Mung bean exhibited chlorotic lesions that became necrotic on primary leaves (Fig. 1D) and mottling on the secondary leaves. Monarch and Crimson cowpea, *V. faba*, and Lee soybean were not susceptible to BGMV.

Physical and chemical properties. The extinction coefficient ($E^{1\%}_{260nm}$) of BGMV was 50.0. The yields of purified BGMV ranged from 75 to 125 mg/kg of bean tissue.

Purified virus preparations exhibited one centrifugal component with a sedimentation coefficient of 122S (Fig. 2). Electron microscopic examination of these preparations showed polyhedral particles with diameters of 28 nm (Fig. 3). Immunoelectrophoresis showed that BGMV consisted of a single electrophoretic component (Fig. 4) that migrated toward the anode.

Density determination runs resulted in a sharp band (Fig. 5). Virion density was 1.364 g/cm^3. With the method of Sehgal et al (15), BGMV was estimated to contain about 20% nucleic acid. After 2–3 days in CsCl, followed by dialysis to remove the salt, stability of the virus was demonstrated by retention of infectivity on Pinto bean.

Approximately 60% of the total nucleic acid was recovered by phenol-SDS extraction. The ratio of maximum to minimum absorbance (A_{260nm}/A_{230nm}) was 2.3. Base ratio was G = 24.9%; A = 25.9%; C = 25.3%; U = 24.0%. The nucleic acid was infectious on Pinto bean and lost all infectivity after incubation with ribonuclease, whereas the infectivity of whole virus was relatively unaffected.

Five components were consistently observed in density gradient centrifuged nucleic acid extracted with phenol-SDS (14). Only the most rapidly sedimenting component was infectious. Polyacrylamide gel electrophoresis of similar preparations also showed several components. With Lane's dissociation buffer (7), however, only one component was observed with either separation technique. The BGMV nucleic acid had a molecular weight of approximately 1.4×10^6 daltons and comigrated with southern bean mosaic virus nucleic acid.

Polyacrylamide gel electrophoresis showed that BGMV has a single protein subunit with a molecular weight of about 38,200 daltons.

Serology. The titer of the BGMV antiserum was 1:1,024 by 8 wk after injections of rabbits were begun. No serologic relationships were found between BGMV and other viruses.

Beetle transmission. Blackgram mottle virus was transmitted by seven of 20 Mexican bean beetles the first day after acquisition feeding. No transmissions occurred after the first day. Virus was transmitted by four of 16, three of 17, one of 20, and one of 14 bean leaf beetles that fed one through four days after acquisition feeding, respectively.

DISCUSSION

Blackgram mottle virus is a typical beetle-transmitted virus in that it is isometric, stable, and highly antigenic (3). Phatak (12) reported that three species of aphids failed to transmit BGMV and

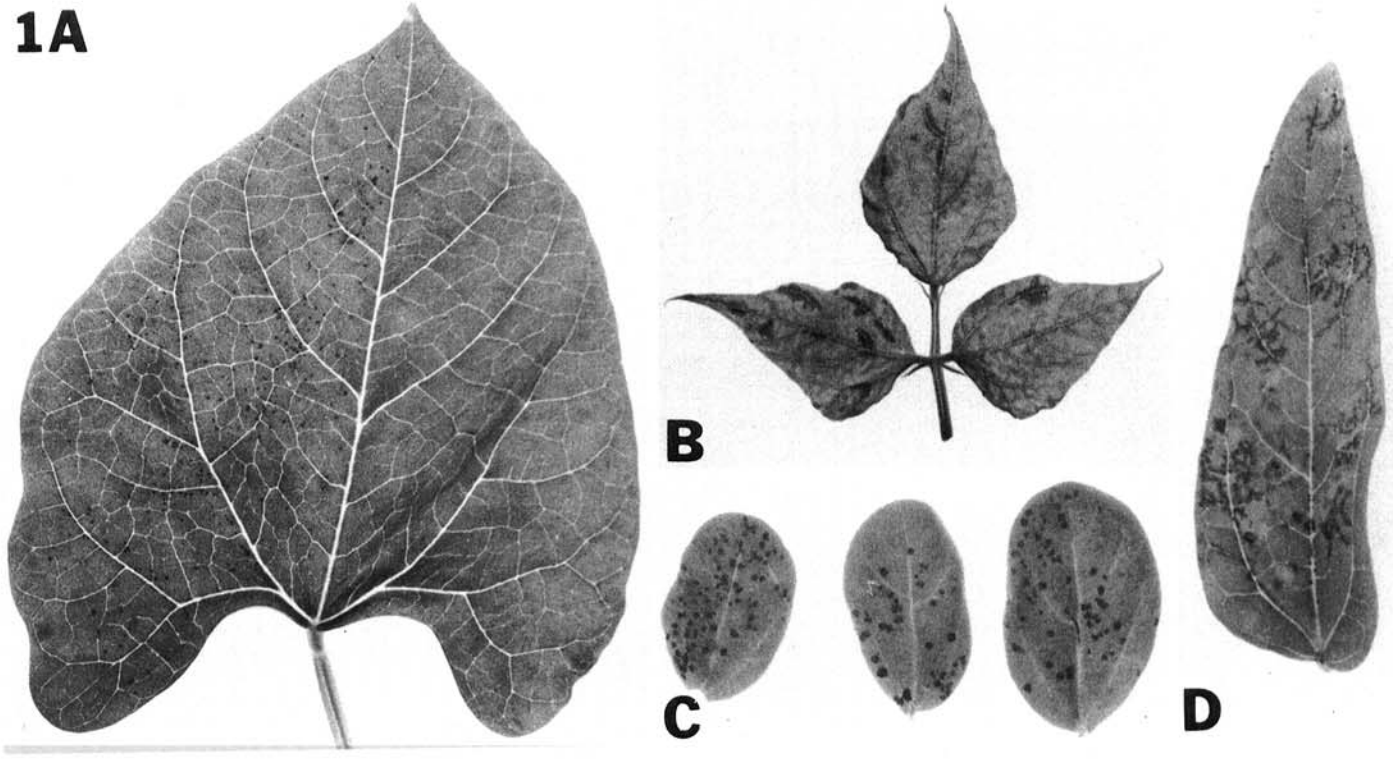

Fig. 1. Symptoms caused by infection with blackgram mottle virus. **A**, Local lesions on left half of leaf of *Phaseolus vulgaris* 'Pinto.' **B**, Mosaic in Black Valentine bean. **C**, Local lesions on cotyledons of *Cyamopsis tetragonoloba* (guar). **D**, Localized necrosis on primary leaves of *Phaseolus aureus* (mung bean).

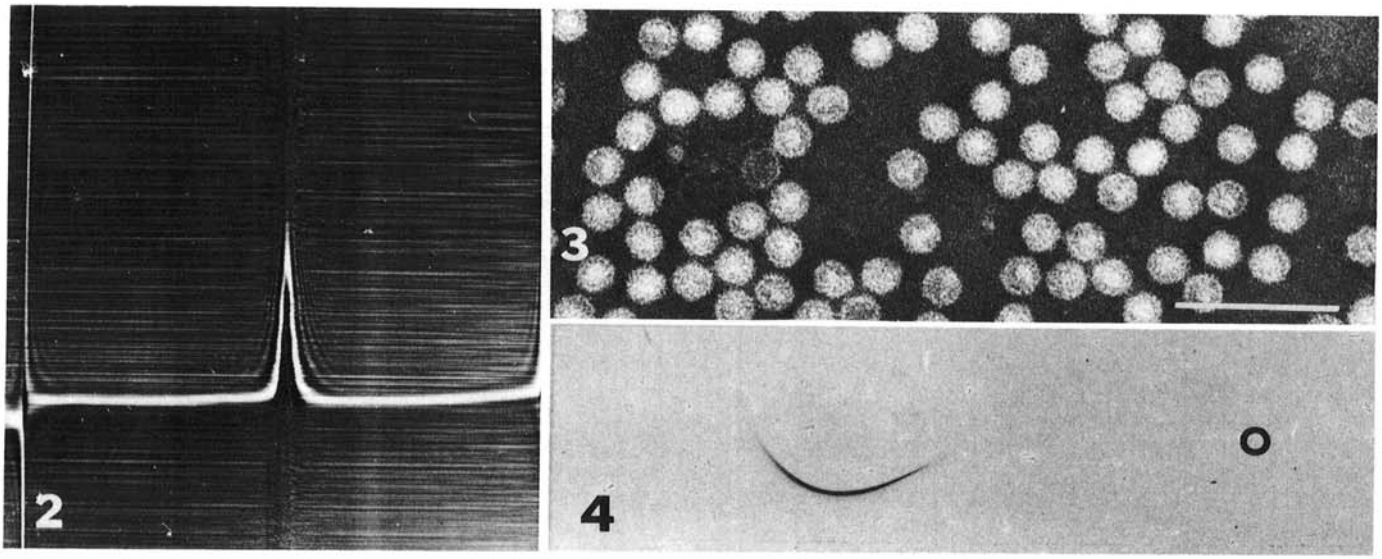

Fig. 2–4. 2, Schlieren sedimentation pattern of purified blackgram mottle virus. Photograph taken 16 min after reaching speed of 32,000 rpm at 20 C. Sedimentation from left to right. 3, Electron micrograph of a purified preparation of blackgram mottle virus negatively stained with 1% sodium phosphotungstate, pH 6.8. Bar represents 100 nm. 4, Immunoelectrophoresis of blackgram mottle virus in 1% agarose in Tris-barbitone buffer, pH 8.8. Anode at left O = origin.

Fig. 5. Densitometer tracing of buoyant density profile of blackgram mottle virus in CsCl after 24-hr centrifugation at 44,000 rpm. Density increases from left to right.

correctly predicted that the vectors of BGMV would be beetles.

Phatak found that the host range of BGMV is limited to legumes and reported that all cowpea varieties and soybean are susceptible (12). *V. faba* showed symptoms but virus could not be recovered. In our study, however, Monarch and Crimson cowpea, Lee soybean, and *V. faba* were not susceptible to BGMV.

This report on BGMV expands the list of beetle-transmitted legume viruses. Fulton et al (3) listed three groups: the comoviruses, the bromoviruses, and southern bean mosaic virus. Recently, Waterworth et al (17) and Shoyinka et al (16) added bean mild mosaic and cowpea mottle viruses, respectively.

Southern bean mosaic, bean mild mosaic, cowpea mottle, and BGMV each exhibit a single centrifugal component and contain about 20% nucleic acid, which has a molecular weight of about 1.4×10^6. The protein subunit molecular weights differ (southern bean mosaic = 26,700 [5], cowpea mottle = 44,500 [16], and BGMV = 38,200), and the four viruses are not serologically related.

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