Isolation and Partial Characterization of a Geminivirus Causing Bean Dwarf Mosaic

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ABSTRACT

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Bean dwarf mosaic virus (BDMV) was transmitted mechanically and by the whitefly *Bemisia tabaci* from and to susceptible bean (*Phaseolus vulgaris*) cultivars. Two species of *Sida*, suspected weed reservoirs of BDMV, were also shown to be susceptible hosts by whitefly transmission tests. Electron microscopy of ultrathin sections of BDMV-infected bean and *Sida* sp. leaf tissue revealed the presence of viruslike particle aggregates in phloem cells. Quasi-isometric nucleoprotein particles ($A_{260/280nm}$ = 1.4), found predominantly in pairs measuring about 20 × 33 nm, were isolated from BDMV-infected bean plants. Purified nucleoprotein was infective to Topcrop bean plants, inducing characteristic bean dwarf mosaic symptoms. Dissociated infectious nucleoprotein yielded a single protein species of molecular mass 27,500 daltons and a single nucleic acid band of approximately 2.6 kb. Purified nucleic acid was resistant to RNase but sensitive to DNase I and nuclease S1, indicating the presence of single-stranded DNA. The isolated virus was serologically related to African cassava mosaic, bean golden mosaic, mung bean yellow mosaic, and tomato golden mosaic viruses. Bean dwarf mosaic virus is proposed to be a new member of the geminivirus group.

Additional keywords: achaparramiento, bean chlorotic mottle, infectious chlorosis of Malvaceae.

The name bean dwarf mosaic (BDM) was originally given to a disease of the common bean (*Phaseolus vulgaris* L.), characterized by severe plant dwarfing and variegation (5). This disease has also been referred to as bean chlorotic mottle and infectious chlorosis of Malvaceae (8). Susceptible bean genotypes infected at the seedling stage usually exhibit dwarfing, leaf malformation, and chlorotic or yellow patches (Fig. 1), whereas moderately susceptible genotypes or plants infected at a later stage may only show the variegated patches irregularly distributed in the foliage (Fig. 2). Witches'-broom symptoms have often been described as part of the BDM syndrome (8), but these malformations may be the result of mixed virus infections (15). BDMV-infected plants generally abort their flowers or produce severely distorted pods (8).

BDM is widely distributed in Latin America at a relatively low incidence (5,8). However, this disease has a considerable epidemiologic potential as observed during 1978–1981, when successive outbreaks of BDM caused the total loss of over 40,000 ha of beans in northwestern Argentina (2).

The etiology of BDM has been investigated by various workers. In 1975, Costa (5) suggested that BDM was caused by a strain of the Abutilon mosaic virus complex, transmitted from malvaceous weeds, mainly *Sida* spp., to bean. Costa (4) had previously reported the transmission of the causal agent from *Sida* spp. to bean by the whitefly *Bemisia tabaci* Genn. Later, Kitajima and Costa (19) demonstrated the presence of isometric particles, 20–25 nm in diameter, in ultrathin sections of *Sida* spp. leaves affected by a bright yellow mosaic. However, several attempts to isolate the agent, or visualize it in infected leaf extracts using electron microscopy, were unsuccessful (4,8). In 1982, Jayasinghe (15) reported the isolation of two beetle-transmitted viruses (bean mild mosaic and bean southern mosaic viruses) and one aphid-borne virus (cucumber mosaic virus) from bean plants with BDM symptoms. A series of mechanical inoculation tests led Jayasinghe to conclude that BDM was the result of mixed infections by different combinations of the three viruses.

This investigation was undertaken, first, in response to the increasing incidence of BDM in important bean-producing regions of Latin America, and secondly, because of the close association that exists between this disease and the presence of the whitefly *B. tabaci* in BDM-affected bean plantings.

MATERIALS AND METHODS

Pathogen source. One-month-old bean plants showing characteristic BDM symptoms, such as dwarfing, leaf distortion, and variegation, were collected from bean fields in Palmira, Valle, Colombia. The samples were analyzed by double immunodiffusion and immunosorbent electron microscopy tests using antisera to bean mild mosaic, bean southern mosaic, and cucumber mosaic viruses. Only those samples that assayed negative in these serological tests and did not contain any anisometric virus particles (as determined by electron microscopy), were selected for this study.

Mechanical inoculation tests. Young trifoliate leaves of BDM-affected bean (*P. vulgaris* 'Topcrop') plants were ground (1:4, w/v) in cold 0.1 M KPO_4 buffer, pH 7.6. The inoculum was applied to Carborundum-dusted (600 mesh) primary leaves of 8-day-old Topcrop and Mochis 440 bean seedlings, using sterile

cotton swabs. Inoculated test plants were maintained in a glasshouse with a maximum light intensity of 1,100 μE m^{-2} sec^{-1}, an average temperature of 27 C, and relative humidity of 75%. Other plant species inoculated mechanically in this study were *Chenopodium amaranticolor* Coste & Reyn., *Glycine max* (L.) Merr. (eight different cultivars), *Lycopersicon esculentum* Mill., *Nicotiana tabacum* L., and *Vigna unguiculata* (L.) Walp.

Insect transmission tests. Adult individuals of the whitefly *Bemisia tabaci*, reared on eggplant (*Solanum melongena* L.), were confined with the aid of small (22 mm in diameter) round cages on the leaves of 18-day-old Topcrop or Mochis 440 bean plants mechanically inoculated as described above. After an overnight acquisition-feeding period, the whiteflies were transferred (10 per test plant) into cages containing virus-free 8-day-old bean seedlings. The whiteflies were maintained on test plants until the final evaluation date (up to 45 days).

Pathogenicity tests. Twenty-four bean cultivars, representing some of the main seed types of *P. vulgaris*, were inoculated mechanically and by the whitefly *B. tabaci* as described above. Also *Sida rhombifolia* L. and *S. spinosa* L., which are considered as potential wild reservoirs of BDMV (8), were selected as test plants and sources of inoculum for transmission tests with the whitefly *B. tabaci*.

Virus purification. The purification procedure involved a modification of the method developed by Jaramillo and Lastra (14) to isolate Euphorbia golden mosaic virus. BDMV-infected Topcrop seedlings were transferred 8 days after mechanical inoculation from the glasshouse to a growth room with an average temperature of 23 C, 75% relative humidity, and average light intensity of 652 μE $m^{-2}sec^{-1}$. After 24 hr, 30 g of trifoliate leaves were harvested and frozen overnight at −80 C. Frozen tissue was crushed manually and homogenized with a blender for 3 min in 60 ml of 0.1 M KPO_4 buffer, pH 7.5, containing 1% 2-mercaptoethanol and 0.1% driselase. The homogenized mixture was stirred at 10 C for 1 hr and then squeezed through three layers of cheesecloth. The filtered extract was centrifuged at 10,400 *g* for 15 min and the supernatant was collected. The supernatant was stirred for 2 hr at 10 C with 4% polyethylene glycol (PEG 8,000), 1% NaCl, and 0.5% Triton X-100 and then centrifuged at 10,400 *g* for 15 min. The pellet was resuspended overnight in 10 ml of 0.01 M KPO_4 buffer, pH 7.5, at 4 C, before clarification by centrifugation at 12,100 *g* for 10 min. The supernatant was subjected to ultracentrifugation at 170,000 *g* for 90 min, and the resulting pellet resuspended in 1 ml of 0.01 M KPO_4 buffer, pH 7.5, for 2 days at 4 C, before centrifugation for 10 min at 12,100 *g*. The clarified suspension was layered onto log-linear sucrose gradients prepared in 0.01 M KPO_4 buffer, pH 7.5, and then centrifuged at 150,000 *g* in a Beckman SW 41 rotor for 270 min. A visible band, located approximately 23 mm from the bottom of the 12-ml tubes, was collected using an ISCO density gradient fractionator, diluted with 0.01 M KPO_4 buffer to a final volume of 10 ml, and then concentrated by ultracentrifugation at 170,000 *g* for 90 min. The pellet was resuspended in 0.5 ml 0.01 M KPO_4 buffer, pH 7.5, for 4 days at 4 C. The resulting suspension was further subjected to equilibrium density gradient centrifugation in a preformed 20–30% Cs_2SO_4 gradient prepared in 0.01 M KPO_4 buffer, pH 7.5, and centrifuged in a Beckman SW 65 rotor at 120,000 *g* for 270 min. A visible zone located approximately 20 mm from the bottom of the 5.5-ml tube was collected in a dropwise manner through a needle hole punched in the bottom of the tube. The collected volume was diluted to 10 ml in 0.01 M KPO_4 buffer, pH 7.5, and concentrated by ultracentrifugation at 170,000 *g* for 90 min. Finally, the pellet was resuspended overnight at 4 C in 0.3 ml of 0.01 M KPO_4 buffer.

Bean golden mosaic virus (BGMV) was purified as described by Morales and Niessen (22).

Buoyant density determinations. Purified virus preparations (0.5 mg/tube) were layered onto 30% (w/w) Cs_2SO_4 solutions prepared in 0.01 M KPO_4 buffer, pH 7.5, and centrifuged at 100,000 *g* for 17 hr in a Beckman SW 65 rotor. The visible virus band, located 28 mm from the bottom of the 5.5-ml tube, was collected with a syringe to determine its refractive index with a Bausch & Lomb Abbe-3L refractometer.

The buoyant density of the virions was calculated according to the equation relating the refractive index to the density of Cs_2SO_4 (11). The buoyant density of the viral nucleic acid was determined for a 200-μl solution containing 20 μg/ml of nucleic acid in 10 mM Tris-HCl, 1 mM ethylene diaminetetraacetic acid (EDTA), pH 8.0. This solution was layered onto a 7-ml, 51% (w/w) CsCl solution prepared in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, in quick-seal tubes, and centrifuged at 170,000 *g* for 19 hr at 20 C. After centrifugation, 200-μl fractions were collected by puncturing the bottom of the tubes for spectrophotometric analysis at 260 nm. Fractions with the highest A_{260nm} value were finally used to determine the refractive index and buoyant density of the nucleic acid, using standard density conversion data (3).

Fig. 1. Plant dwarfing exhibited by a sensitive bean genotype affected by bean dwarf mosaic.

Fig. 2. Characteristic mosaic and foliar distortion symptoms observed in moderately susceptible bean genotypes affected by bean dwarf mosaic.

Spectrophotometry. Absorption spectra of purified preparations were obtained with a Beckman DU 50 spectrophotometer. The extinction coefficient of 7.7 cm^2mg^{-1} at 261 nm, determined for bean golden mosaic virus (10), was used to estimate the concentration of purified virus preparations.

Cytology. Tissue sections obtained from BDMV-infected Topcrop bean and *Sida spinosa* plants were prepared for ultramicrotomy by preliminary fixation with half-strength Karnovsky fixative (16) for 24 hr at 4 C. The glutaraldehyde used to prepare the Karnovsky fixative had been previously agar-purified following the method described by Nicklas et al (23). After dehydration in a graded series of alcohol and acetone, the fixed tissue was embedded in the low-viscosity epoxy resin medium recommended by Spurr (27). Thin sections were obtained with a MT6000 Sorvall ultramicrotome.

Electron microscopy. For observation of partially purified or purified virus preparations, Formvar-coated and carbon-shadowed 200-mesh copper grids were incubated for 30 min with 1% aqueous glutaraldehyde. Excess glutaraldehyde was washed with 0.01 M KPO_4 buffer and, then, a drop of the virus preparation was placed on the grid for 30 min. The grid was washed with distilled water and stained with 2% aqueous uranyl acetate. Thin sections of BDMV-infected bean tissues were stained with uranyl acetate and Reynolds lead citrate (24). All samples prepared for electron microscopy were observed with a JEOL 100 SX electron microscope.

Polyacrylamide gel electrophoresis. *Protein analysis.* The electrophoretic analysis of purified virus preparations in polyacrylamide gels containing SDS (SDS-PAGE) was basically performed as described by Weber and Osborn (29). Electrophoresis was carried out in 7.5% acrylamide gels using a vertical slab cell. Samples were dissociated in two volumes of a solution containing 0.01 ml of the $NaPO_4$ buffer used for electrophoresis, 0.25 ml of 10% SDS, 0.025 ml of 2-mercaptoethanol, and 0.25 ml of 60% sucrose, followed by boiling for 1 min. Serum albumin (67 kDa), carbonic anhydrase (29 kDa), bean golden mosaic virus coat protein (26 kDa), and bean mild mosaic virus coat protein (46 kDa) were used as markers for molecular mass determinations.

Nucleic acid analysis. Viral nucleic acids were isolated by multiple extractions with phenol:chloroform:isoamyl alcohol (25:24:1, by volume). The aqueous phase was adjusted to 0.2 M sodium acetate, pH 5.0, 2.0 volumes of ethanol were added, and the solution was incubated overnight at −20 C. Nucleic acids were recovered by centrifugation and resuspended in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0.

Size estimation was carried out by electrophoresis of nucleic acid extracts obtained from purified BDMV and BGMV preparations, run separately in 1.0% agarose gels (89 mM Tris-borate, 89 mM boric acid, 2 mM EDTA, pH 8.0) for 2 hr at 80 V. Gels were stained with 0.1 μg/ml ethidium bromide. The BDMV and BGMV bands were subsequently transferred to nitrocellulose membranes following the standard Southern blot procedure (20). An M-13 derivative containing a 1,650-bp fragment of BGMV DNA 1 (produced by Dr. S. Haber, University of Illinois) was used as a probe. This probe was labeled by nick translation as described by Maniatis (20). Prehybridization was carried out in 6× saline sodium citrate (SSC), 5× Denhardt's solution, 1% SDS, and 100 μg/ml of sheared salmon sperm DNA for 2 hr at 60 C. Hybridization with the BGMV DNA-1 probe was performed in the prehybridization buffer at 55 C for 16 hr. Blots were washed once with 2× SSC for 10 min at room temperature, twice with 0.5× SSC for 15 min at 55 C, and once with 0.5× SSC for 10 min at room temperature, always in the presence of 0.5% SDS. Autoradiography was done with Kodak X-Omat AR film, using a Dupont Cronex Lightning-Plus intensifying screen, at −70 C for 10 hr.

To determine the nature of the BDMV genome, aliquots of 0.5 μg of isolated BDMV nucleic acid were digested with one of three enzymes: 1) DNase I (RNase-free DNase, Sigma) at enzyme/nucleic acid ratios of 2, 5 and 10 (w/w) in 10 mM Tris-HCl, pH 7.8, containing 10 mM $MgCl_2$; 2) RNase A (protease-free RNase IA Sigma, pretreated by heating at 96 C for 15 min) at enzyme/nucleic acid ratios of 20, 50, and 100 (w/w) in 10 mM Tris-HCl, 1 mM EDTA, pH 7.8; and 3) 25 or 50 units of nuclease S1 (nuclease S1, type III, Sigma) in 10 mM NaOAc, pH 5.3, containing 0.5 mM $ZnCl_2$. After incubation at 37 C for 1 hr, the reactions were stopped by adjusting solutions to 0.1% SDS, 10 mM EDTA, and heating at 95 C for 15 min (1,13). The products of the reaction were phenol-extracted, ethanol-precipitated, mixed with 0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol, and run in a 1.6% agarose gel (0.089 M Tris-borate, 0.089 M boric acid, 0.002 M EDTA, pH 8.0) for 5 hr at 80 V. Gels were stained in 1 μg/ml of ethidium bromide and observed with an ultraviolet light transilluminator.

Serology. *Antiserum production.* An antiserum was prepared by injecting a New Zealand white rabbit with purified infective nucleoprotein preparations obtained from BDMV-infected Topcrop bean plants, standardized to a concentration of approximately 1.4 mg/ml. A series of three injections were given at weekly intervals, using the foot pad technique of immunization (30). Each injection consisted of 0.25 ml of the purified preparation emulsified with an equal volume of Freund's complete (first injection) or incomplete (subsequent injections) adjuvant. The antiserum was collected at weekly intervals for a month after the last injection.

Antisera to the following geminiviruses were kindly sent to CIAT by those named: African cassava mosaic virus (B. D. Harrison), mung bean yellow mosaic virus (Y. Honda), and tomato golden mosaic virus (K. W. Buck). The antisera to bean golden, bean mild, and bean southern mosaic viruses were previously produced at CIAT (F. Morales, A. Niessen, and M. Castaño, *unpublished*). Antiserum to cucumber mosaic virus was kindly provided by D. E. Purcifull.

Double immunodiffusion tests. Ouchterlony tests were performed in medium containing 1.0% Noble agar, 0.8% NaCl, and 0.1% sodium azide prepared in 0.01 M Tris-HCl, pH 7.6 (w/v). The antigen was obtained by homogenizing 1 g of BDMV-infected bean leaf tissue in 0.5 ml of distilled water with a mortar and pestle. Leaf extracts were centrifuged for 15 min in an Eppendorf microcentrifuge, and the resulting supernatants were pipetted into their respective antigen wells. Leaf extracts from bean plants infected with BGMV and from healthy bean plants were used as controls. Either BGMV or BDMV antisera were placed in the center wells, and the plates were incubated overnight at 24 C before the final recording of results.

Immunosorbent electron microscopy (ISEM). Formvar-coated, carbon-shadowed 200-mesh copper grids were incubated at room temperature for 1 hr with a drop of a 1:50 dilution of either BDMV or BGMV antisera in 0.01 M KPO_4 buffer, pH 7.5. Young trifoliate leaves (about 0.1 g) detached from BDMV-infected Topcrop plants were ground in approximately 200 μl of 0.01 M KPO_4 buffer, pH 7.5, and the extracts centrifuged in an Eppendorf microcentrifuge for 15 min. The antiserum-coated grids were washed with 0.01 M KPO_4 buffer, pH 7.5, and then floated on a drop of centrifuged leaf extract for 4 hr at 4 C. Treated grids were stained with a drop of 2% aqueous uranyl acetate for 2 min. Grids were observed at 50,000× using a JEOL 100 SX electron microscope.

RESULTS

Mechanical transmission. BDMV was sap-transmitted from and to Topcrop bean plants with up to 100% efficiency. Most of the systemically infected Topcrop bean plants developed characteristic dwarf mosaic symptoms within 8 days after inoculation. The efficiency of mechanical transmission, however, varied considerably among the different bean cultivars tested, and some could not be infected in repeated tests (Table 1). None of the other mechanically inoculated plant species tested expressed symptoms. The bean cultivar Alubia proved highly susceptible in both the whitefly and mechanical transmission tests, and plants inoculated at an early stage of development exhibited severe dwarfing.

Whitefly-transmission tests. BDMV was efficiently transmitted by *B. tabaci* from and to Topcrop bean plants (up to 100%). BDMV was also transmitted by *B. tabaci* from infected Topcrop bean plants to *Sida spinosa*, and from *S. spinosa* to Topcrop bean and *S. rhombifolia*. A bright golden mosaic developed in systemically infected *Sida* spp. plants within a month after inoculation. Topcrop bean plants exhibited characteristic dwarf mosaic symptoms within 10 days of their exposure to viruliferous whiteflies. BDMV was transmitted by *B. tabaci* to all the bean cultivars shown to be susceptible by mechanical inoculation, as well as to eight other cultivars that could not be infected (serologically negative) in the mechanical transmission tests (Table 1). Three of these eight cultivars (ICTA-Quetzal, Porrillo Sintetico, and Red Mexican 34) reacted with only a few localized chlorotic leaf spots (1–3/plant). Plants exhibiting these chlorotic lesions were shown by ISEM to contain isometric viruslike particles in the local lesions but not in symptomless leaves.

Physico-chemical properties. The purification procedure described here produced a concentrated suspension of quasi-isometric viruslike particles about 20 nm in diameter, found predominantly in pairs forming bisegmented structures measuring about 20 × 33 nm (Fig. 3). Spectrophotometric analyses of these purified preparations revealed an ultraviolet light-absorbing spectrum characteristic of a nucleoprotein, with an average $A_{260/280nm}$ value of 1.4. These purified preparations were infectious to mechanically inoculated Topcrop bean plants and induced characteristic dwarf mosaic symptoms. The yield of the nucleoprotein isolated varied between 6.4 and 11.4 mg/kg of infected bean tissue (uncorrected for light scattering). The estimated buoyant densities of the virion and viral nucleic acid were 1.2999 and 1.7095 g/cm^3, respectively.

Cytology. Electron microscopy of ultrathin sections of BDMV-infected bean tissue revealed the occurrence of distinct cytopathological abnormalities in the nuclei of phloem parenchyma cells. The most noticeable cellular alterations were the presence of fibrillar bodies and viruslike particle aggregates in the nucleus of phloem parenchyma cells (Fig. 4). Similarly, ultrathin sections of BDMV-infected leaf tissue of *S. spinosa* were shown by electron microscopy to contain viruslike particle aggregates in the nucleus of phloem parenchyma cells (Fig. 5).

Protein and nucleic acid analyses. A single protein species of apparent molecular mass 27,500 daltons was detected by SDS-PAGE of purified BDMV preparations. A single band was observed in agarose gels of nucleic acid extracts from purified BDMV. This band comigrated with the nucleic acid fraction

TABLE 1. Reaction of selected bean cultivars to bean dwarf mosaic virus inoculated mechanically and by the whitefly *Bemisia tabaci*

Cultivar	Inoculation	
	Mechanical	Whitefly
Alubia	+++[a]	+++
Amanda	++	++
Black Turtle Soup	−	−
Cacahuate 72	++	++
Dubbele Witte	++	++
Great Northern 31	−	+
Great Northern 123	−	+
ICA-Pijao	−	−
ICTA-Quetzal	−	+/−
Improved Tendergreen	++	NT
Imuna	+++	+++
Jubila	+	++
Michelite	−	+
Monroe	−	+
Pinto 114	−	−
Porrillo Sintetico	−	+/−
Red Kloud	++	NT
Red Mexican 34	−	+/−
Red Mexican 35	−	−
Redlands Greenleaf B	+	+
Redlands Greenleaf C	+	+
Sanilac	−	+
Stringless Green Refuge	++	++
Topcrop	+++	+++
Widusa	+	+

[a] + = Susceptible, but more than 60% of the inoculated test plants escaped infection (serologically tested); ++ = susceptible and at least 50% of the inoculated plants were infected; +++ = susceptible with over 70% of the inoculated test plants being infected; +/− = serologically detected only in localized lesions; − = immune; NT = not tested.

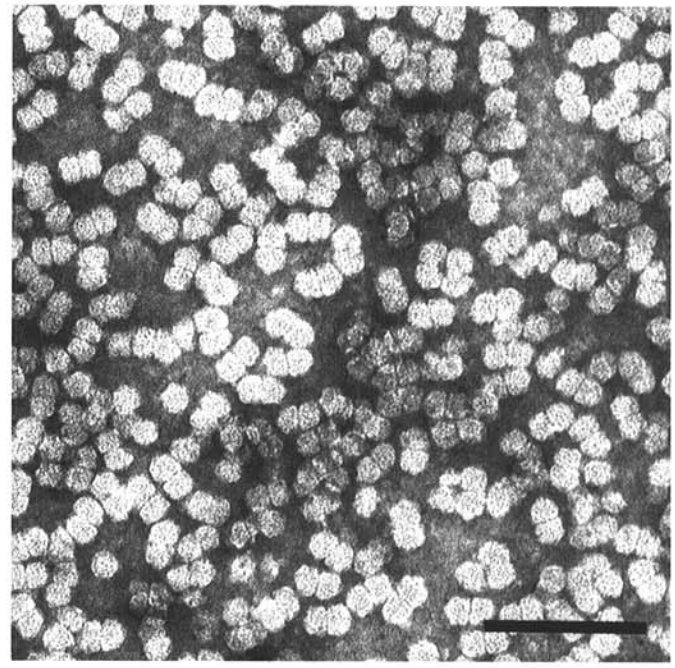

Fig. 3. Electron micrograph of purified bean dwarf mosaic virus negatively stained in 2% uranyl acetate. Scale bar = 0.1 μm.

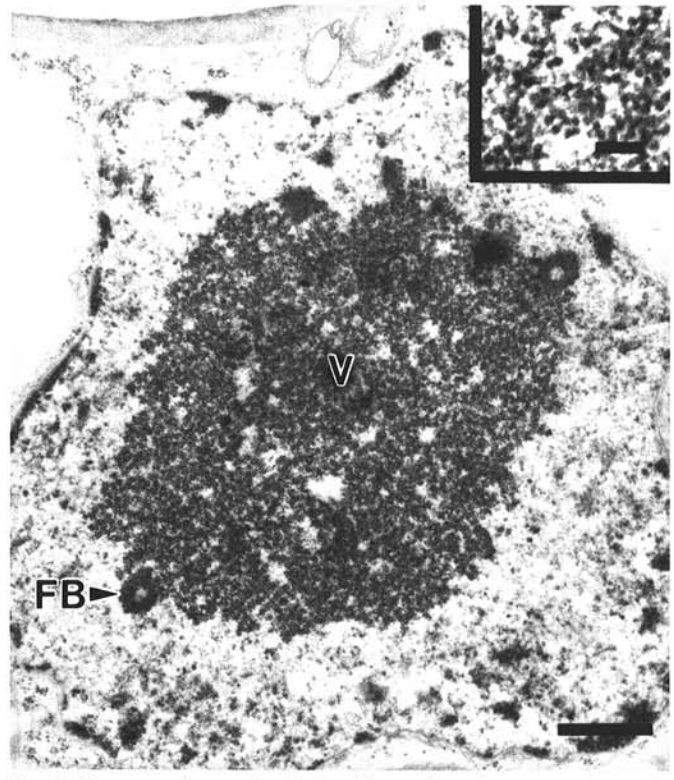

Fig. 4. Electron micrograph of ultrathin section of bean dwarf mosaic virus-infected Topcrop bean leaf tissue showing fibrillar bodies (FB) and viruslike particle aggregate (V) in the nucleus of an infected phloem parenchyma cell. Scale bar = 1 μm. Inset shows magnification of the virus particle aggregate. Scale bar = 0.1 μm.

obtained from purified BGMV (Fig. 6A), which consists of two ssDNA molecules, each about 2.6 kb (12). The nucleic acid band extracted from purified BGMV specifically hybridized with the BGMV DNA-1 probe (Fig. 6B). The BDMV nucleic acid was resistant to RNase but sensitive to degradation by DNase I and nuclease S1 (Fig. 7), indicating the presence of single-stranded DNA.

Serology. In gel diffusion tests, positive precipitation reactions were observed between wells containing antiserum to BDMV and those containing purified BDMV preparations obtained from infected Topcrop bean plants. In reciprocal gel diffusion tests, BDMV was antigenically indistinguishable (no spur formation) from BGMV. In ISEM tests, however, coating of grids with BDMV antiserum produced a 46-fold and 17-fold increase in the number of BDMV and BGMV particles trapped, respectively, as compared with nontreated control grids. Similarly, when BGMV antiserum was used in ISEM tests, 118-fold and 16-fold increases were recorded for BGMV and BDMV, respectively. The other three geminivirus antisera tested also gave increases of three-fold or more in the number of BDMV particles trapped, which is considered as evidence of serological relationship (26).

DISCUSSION

The results obtained in this investigation demonstrate that BDM is caused by a whitefly-borne member of the geminivirus group (9,10,12,21). The BDMV isolate selected for this study was mechanically transmissible, but as reported (6,7) for BGMV, some BDMV isolates collected during this study could not be mechanically transmitted (F. Morales, *unpublished*). BDMV has a relatively wide pathogenicity range in *P. vulgaris*, as demonstrated here in the whitefly inoculation tests with different bean cultivars. However, some bean genotypes, such as Pinto 114 and Red Mexican 35, should be quite valuable as sources of BDMV resistance in future breeding projects. The high BDMV susceptibility of the bean cultivar Alubia partly explains the severe outbreaks of BDMV in northwestern Argentina, where Alubia was the predominant bean cultivar before the appearance of this virus.

The bisegmented virus particles and nuclear inclusions observed by electron microscopy of purified preparations and BDMV-infected bean cells, respectively, are characteristic features of plant infections caused by geminiviruses (9,12,17,18,21). Also, the ultraviolet light absorption spectrum, buoyant density, molecular

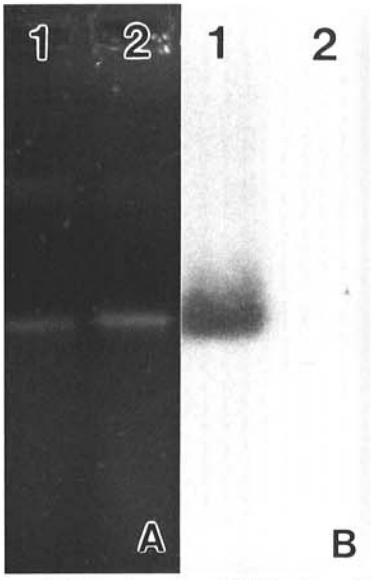

Fig. 6. A, Agarose gel electrophoresis and **B,** Southern-blot hybridization analysis with a bean golden mosaic virus (BGMV) probe of nucleic acids isolated from purified BGMV and bean dwarf mosaic virus (BDMV) particles. Lanes 1 and 2 are BGMV and BDMV, respectively.

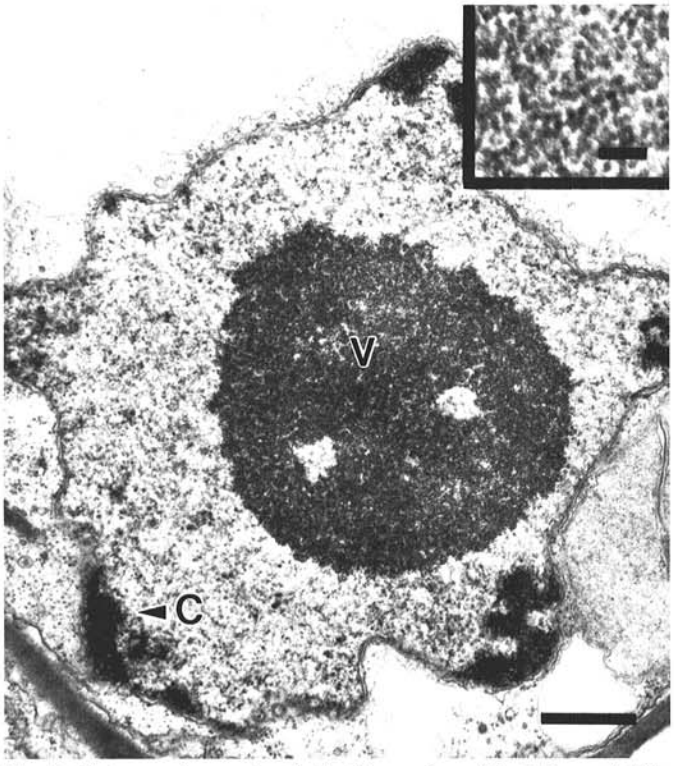

Fig. 5. Electron micrograph of ultrathin section of leaf tissue of *Sida spinosa* infected by bean dwarf mosaic virus, showing virus particle aggregate (V) and chromatin (C) in the nucleus of a phloem parenchyma cell. Scale bar = 1 μm. Inset shows magnification of virus particle aggregate. Scale bar = 0.1 μm.

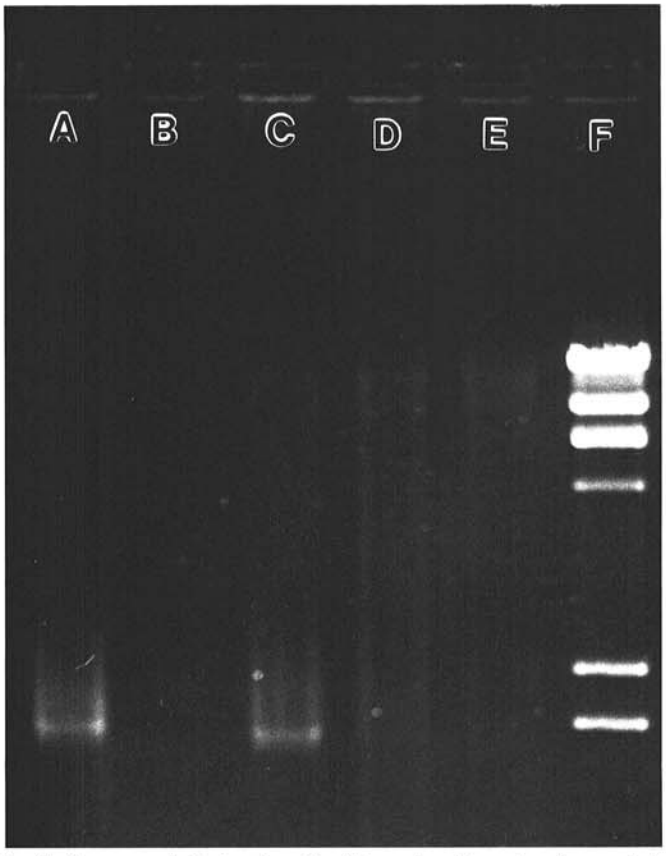

Fig. 7. Agarose gel electrophoresis of bean dwarf mosaic virus nucleic acid stained with ethidium bromide. Lane A, untreated; B, DNase-I-treated; C, RNase-treated; D, nuclease S1-treated (25 units); E, nuclease S1-treated (50 units); F, lambda DNA *Hin*dIII fragments (included to determine the relative position of viral nucleic acid only).

weight, and other physico-chemical properties of the virion and/or virion-associated protein and nucleic acid of BDMV are in close agreement with similar data published for other geminiviruses (9,10,12).

As reported for other whitefly-transmitted geminiviruses (25,26,28), BDMV was also serologically related to three whitefly-borne geminiviruses isolated from different plant species. It is evident that BDMV is serologically related to BGMV, but the results of the reciprocal ISEM tests conducted in this study also revealed appreciable antigenic differences between BDMV and BGMV (10). Furthermore, BDMV can be distinguished from BGMV by the different symptoms these two viruses induce in susceptible bean genotypes and by notable differences in their pathogenicity range. In general terms, BDMV tends to induce severe plant stunting, and the distribution of mosaic symptoms in systemically infected bean plants is restricted and irregular in comparison with the more uniform systemic yellowing induced by BGMV. With respect to their pathogenicity range in *P. vulgaris,* none of the bean genotypes inoculated with BDMV in this investigation was immune to BGMV in a previous study conducted under similar experimental conditions (22). Also, previous attempts to infect BDMV-susceptible *Sida* species with BGMV have given negative results (5). Finally, the BGMV DNA-1 probe used in this investigation did not hybridize with the BDMV genome under high stringency conditions.

Interestingly, some of the bean genotypes inoculated here with BDMV (Red Mexican 34, ICTA-Quetzal, and Porrillo Sintetico) appear to have the capacity to restrict the systemic spread of the virus in plants exposed to viruliferous whiteflies. This phenomenon is currently under investigation to find out whether BDMV can invade these bean genotypes systemically under certain experimental conditions or at a later stage of plant development.

Considering the notable differences in symptom expression, geographical distribution, pathogenicity, and other physico-chemical properties that exist between BDMV and BGMV, it is proposed here that BDMV be considered as a distinct bean geminivirus. Also, the original name, "bean dwarf mosaic" ("mosaico enano" in Spanish, and mosaico anão in Portuguese), should be retained in reference to the bean disease known as "achaparramiento" (dwarfing) in Argentina, and "moteado clorótico" (chlorotic mottle) elsewhere.

LITERATURE CITED

1. Abuzid, A., and Leske, H. 1986. The purification and characterization of geminiparticles from Abutilon mosaic virus infected Malvaceae. J. Phytopathol. 115:344-353.
2. Centro Internacional de Agricultura Tropical. 1982. Bean Program. 1981 Annu. Rep., Cali, Colombia. 198 pp.
3. Chambers, J. A., and Rickwood, D. 1978. Separation of macromolecules by isopycnic centrifugation in different media. Pages 117-133 in: Centrifugation: A Practical Approach. D. Rickwood, ed. Inf. Retr. Ltd., Washington, DC. 224 pp.
4. Costa, A. S. 1955. Studies on Abutilon mosaic in Brazil. Phytopatol. Z. 24:97-112.
5. Costa, A. S. 1975. Increase in the populational density of *Bemisia tabaci*, a threat of widespread virus infection of legume crops in Brazil. Pages 27-49 in: Tropical Diseases of Legumes. J. Bird and K. Maramorosch, eds. Academic Press, New York. 171 pp.
6. Costa, A. S. 1976. Whitefly-transmitted plant diseases. Annu. Rev. Phytopathol. 14:429-449.
7. Figueira, A., and Costa, A. S. 1986. Tentativas de transmissão mecanica do virus do mosaico dourado do feijoeiro do Brasil. Fitopatol. Bras. 11:433-442.
8. Galvez, G. E., and Cardenas, M. R. 1980. Whitefly transmitted viruses. Pages 263-289 in: Bean Production Problems. H. F. Schwartz and G. E. Galvez, eds. Cent. Int. Agric. Trop., Cali, Colombia. 424 pp.
9. Goodman, R. M. 1981. Geminiviruses. J. Gen. Virol. 54:9-21.
10. Goodman, R. M., and Bird, J. 1978. Bean golden mosaic virus. No. 192 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 4 pp.
11. Griffith, O. M. 1979. Techniques of preparative, zonal, and continuous flow ultracentrifugation. 3rd ed. Beckman Instruments, Inc., Palo Alto, CA. 50 pp.
12. Harrison, B. D. 1985. Advances in geminivirus research. Annu. Rev. Phytopathol. 23:55-82.
13. Ikegami, M., Haber, S., and Goodman R. M. 1981. Isolation and characterization of virus-specific double-stranded DNA from tissues infected by bean golden mosaic virus. Proc. Natl. Acad. Sci. USA. 78:4102-4106.
14. Jaramillo, S., and Lastra, R. 1986. Purification and properties of the geminivirus Euphorbia mosaic virus. J. Phytopathol. 115:193-203.
15. Jayasinghe, U. 1982. Chlorotic mottle of bean (*Phaseolus vulgaris* L.). Ph.D. thesis. Agric. Univ. Wageningen, Netherlands. 156 pp.
16. Karnovsky, M. J. 1965. A formaldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27:137 A.
17. Kim, K. S., and Flores, E. M. 1979. Nuclear changes associated with Euphorbia mosaic virus transmitted by the whitefly. Phytopathology 69:980-984.
18. Kim, K. S., Shock, T. L., and Goodman, R. M. 1978. Infection of *Phaseolus vulgaris* by bean golden mosaic virus: Ultrastructural aspects. Virology 89:22-33.
19. Kitajima, E. W., and Costa, A. S. 1974. Microscopia electronica de tecidos foliares de plantas afetadas por virus transmitidos por mosca-branca. VII Reun. Anu. Soc. Bras. Fitopatol. 9:54-55.
20. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
21. Matthews, R. E. F. 1979. Classification and nomenclature of viruses. Intervirology 12:129-296.
22. Morales, J. F., and Niessen, A. I. 1988. Comparative responses of selected *Phaseolus vulgaris* germ plasm inoculated artificially and naturally with bean golden mosaic virus. Plant Dis. 72:1020-1023.
23. Nicklas, R. B., Kubai, D. F., and Hays, T. S. 1982. Spindle microtubules and their mechanical associations after the micromanipulation in anaphase. J. Cell Biol. 95:91-104.
24. Reynolds, E. S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. 17:208.
25. Roberts, I. M., Robinson, D. J., and Harrison, B. D. 1984. Serological relationships and genome homologies among geminiviruses. J. Gen. Virol. 65:1723-1730.
26. Sequeira, J. C., and Harrison, B. D. 1982. Serological studies on cassava latent virus. Ann. Appl. Biol. 101:33-42.
27. Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Utrastruct. Res. 26:31.
28. Thomas, J. E., Massalski, P. R., and Harrison, B. D. 1986. Production of monoclonal antibodies to African cassava mosaic virus and differences in their reactivities with other whitefly-transmitted geminiviruses. J. Gen. Virol. 67:2739-2748.
29. Weber, K., and Osborn, M. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244:4406-4412.
30. Ziemiecki, A., and Wood, K. R. 1975. Serological demonstration of virus-specific proteins associated with cucumber mosaic virus infection of cucumber cotyledons. Physiol. Plant Pathol. 7:171-177.