Differentiation of Bean-Infecting Geminiviruses by Nucleic Acid Hybridization Probes and Aspects of Bean Golden Mosaic in Brazil

R. L. GILBERTSON, S. H. HIDAYAT, and R. T. MARTINEZ, Department of Plant Pathology, and S. A. LEONG, Agricultural Research Service, U. S. Department of Agriculture, Plant Disease Resistance Unit, University of Wisconsin-Madison, Madison 53706; J. C. FARIA, EMBRAPA, Centro Nacional de Pesquisa Arroz-Feijão, Goiania, Goiás, 74,000, Brazil; F. MORALES, Centro International de Agricultura Tropical (CIAT), Apartado Aereo 6713, Cali, Colombia; and D. P. MAXWELL, Department of Plant Pathology, University of Wisconsin-Madison, Madison 53706

ABSTRACT

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A bean golden mosaic geminiviral isolate from Goiania, Goiás, Brazil (BGMV-BZ), was determined to be transmitted by whiteflies and induced golden mosaic symptoms and diagnostic geminiviral inclusion bodies and ultrastructural abnormalities in infected bean leaves. In contrast to BGMV isolates from Central America and the Caribbean, BGMV-BZ could not be sap-transmitted to beans. Cloned geminiviral DNA components were used as DNA probes for the rapid and specific detection of BGMV-BZ and three sap-transmissible bean-infecting geminiviral isolates—BGMV from Guatemala (BGMV-GA) and the Dominican Republic (BGMV-DR) and bean dwarf mosaic geminivirus from Colombia (BDMV-CO). A general DNA probe detected all four viral isolates, whereas specific probes detected BGMV-BZ, BDMV, or BGMV-GA and BGMV-DR. Nucleic acid dot and squash blot methods were used to prepare samples for hybridization, and the dot blot method was used to determine relative differences in viral nucleic acid titers in infected bean leaves. The general and specific probes were employed to study the variability of BGMV isolates and potential weed reservoirs of geminiviruses in the Dominican Republic.

Bean golden mosaic geminivirus (BGMV) is a virus transmitted by whiteflies (*Bemisia tabaci* Gennadius) that posseses a single-stranded DNA genome, infects common bean (*Phaseolus vulgaris* L.) and certain other legumes, and occurs throughout Central and South America and the Caribbean (13). Bean golden mosaic (BGM) is characterized by a striking yellow-green mosaic pattern on infected leaves and by stunted and distorted growth. This disease can cause up to 100% yield loss and seriously diminishes the quality of surviving harvestable seed (13). The first report of BGM was by A. S. Costa in 1960 in the state of São Paulo, Brazil (7). Though first considered a disease of minor importance, BGM has spread rapidly and has now been reported to cause significant losses in at least 12 Latin American countries (13). In Brazil, BGM is the most important disease of beans during the dry season and has eliminated commercial bean production in some traditional bean-growing regions, such as areas in the states of Minas Gerais and Paraná (10). Commercial bean production in the dry season in the western region of the Dominican Republic is also threatened by BGM (F. Saladin, *personal communication*).

Recent evidence indicates that genetic variation exists among geminiviruses causing BGM (15,16) and that this variation has been, and will continue to be, a major complication in ongoing attempts to control BGM by classical plant breeding methods. Two bean lines developed by scientists from the Centro Internacional Agricultura Tropical (CIAT), A429 and DOR303, which are moderately resistant (moderate symptoms and acceptable yield) to BGM in Guatemala (25), developed severe BGM symptoms in Brazil (J. C. Faria, *unpublished*). Moreover, BGMV isolates from Central America, Puerto Rico, and the Caribbean are transmissible via plant sap (13,25), whereas isolates from Brazil are not (7,11). Additional genetic variation in bean-infecting geminiviruses is demonstrated by the recent report of bean dwarf mosaic geminivirus (26).

As part of a study on the genetic diversity of bean-infecting geminiviruses throughout South America, Central America, and the Caribbean, experiments were conducted at the Centro Nacional de Pesquisa Arroz-Feijão (CNPAF), Goiânia, Goiás, Brazil, to determine the transmission of the geminivirus(es) associated with BGM. Light and electron microscopy were used to determine the cytopathology of infected leaves and to diagnose viruses. Results indicated that BGM is induced by a geminivirus transmitted by whiteflies that is not sap-transmissible. It also became apparent that methods for rapid detection and differentiation of bean-infecting geminiviruses are needed to adequately investigate genetic diversity among these viruses.

DNA probes have been used for detection of many plant viruses and viroids (2,24,28,32), including geminiviruses (8,18, 27,29,30). Dot blot and squash blot methods have been developed that allow for the efficient preparation of samples for hybridization with nucleic acid probes. Dot blot hybridization has been used to detect viral nucleic acids in animal and plant tissues (2,4,24,28–30) and was quantitative for viral nucleic acids of cauliflower mosaic caulimovirus in plant sap (24). Squash blot hybridization was first used to detect DNA sequences in root tip segments (19) and in Drosophila flies (33) and has more recently been adapted to detect the mycoplasmalike organism that causes Western X-disease (21), the plant pathogenic bacterium *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye (17), and tomato yellow leaf curl geminivirus (27).

In this report, we describe the results of studies conducted with a BGMV isolate from Brazil (BGMV-BZ) and the detection of BGMV-BZ and other bean-infecting geminiviruses by nucleic acid dot and squash blot hybridization methods with cloned geminiviral DNA as probes. These methods were used to detect differences in viral nucleic acid titer in bean cultivars infected with geminiviruses, to study the potential genetic diversity of BGMV in the Domin-

Present address of first author: Department of Plant Pathology, University of California-Davis, Davis 95616.

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ican Republic, and to determine weed reserviors of geminiviruses in the Dominican Republic.

MATERIALS AND METHODS

Virus isolates. The three BGMV isolates used—BGMV-Guatemala (BGMV-GA isolate O) and BGMV-Dominican Republic (BGMV-DR)—were mechanically transmitted via plant sap from bean plants with golden mosaic symptoms collected from Monjas, Guatemala, and San Juan de la Maguana, the Dominican Republic, respectively, and maintained in beans (cv. Topcrop) by sap transmission (25). BGMV-BZ was transmitted by whiteflies from bean plants with golden mosaic symptoms collected at CNPAF and maintained in Topcrop beans by whitefly transmission. Bean dwarf mosaic geminivirus-Colombia (BDMV-CO) was mechanically transmitted via plant sap from infected beans collected near Cali, Colombia, and was maintained in beans by sap transmission (26). Geminivirus-infected plant tissue was imported under APHIS permits PPQ 549 and 573.

Sap transmission. Young infected trifoliolate leaves and leaf buds were collected, frozen in liquid nitrogen in a mortar, and ground into a fine powder with a pestle. The mortar was placed on ice and, for experiments in Brazil, a sap inoculum was produced by adding a 1% suspension of magnesium trisilicate in 0.1 M potassium phosphate buffer (pH 8.0) (1:4 w/v) and a small amount of 600-mesh Carborundum and grinding thoroughly with a pestle. For experiments conducted in Madison, WI, only the potassium phosphate buffer was used. The sap suspension was immediately inoculated onto the entire adaxial leaf surface of 7- to 10-day-old bean seedlings (cv. Topcrop) that had been dusted with 600-mesh Carborundum, by dipping the base of the pestle into the sap and rubbing leaves in a circular motion. The youngest three leaves of *Nicotiana benthamiana* Domin plants at the five- to seven-leaf stage were similarly inoculated. Control treatments were plants inoculated with buffer and Carborundum and, for experiments conducted in Brazil, plants were exposed to viruliferous whiteflies. Plants were maintained in a growth chamber (28 C, 16-hr photoperiod) in Brazil and Madison or in a whitefly-free greenhouse in Brazil, except for plants exposed to viruliferous whiteflies, which were in a separate growth chamber or greenhouse. Results were recorded 17 days after inoculation.

Light and electron microscopy of bean leaves. Leaves were prepared for staining in azure-A, which stains nucleic acids, by removing the cuticle and epidermal tissues by gently rubbing leaves with 600-mesh sandpaper (5). Areas of abraded leaves (3–5 mm^2) were excised and placed in 2-methoxyethanol for 15–30 min to dissolve chlorophyll and then placed in 0.1% azure-A in 0.01 M Na_2HPO_4 for 15–30 min. The tissue pieces were destained in 95% ethanol for 5–15 min, followed by 2-methoxyethyl acetate for 15–30 min, blotted dry, placed on a drop of Euparol (Carolina Biological Supply, Burlington, NC) on a glass slide, and covered with a coverslip. Stained tissue pieces were viewed with a light microscope at various magnifications.

Bean leaves infected with the virus, which had abundant viral inclusion bodies, and uninfected leaves were selected for examination by electron microscopy. Tissue pieces (3 mm^2) were fixed in 5% glutaraldehyde in 0.08 M cacodylate buffer, pH 7.4, and postfixed in 2% osmium tetroxide in phosphate buffer (9). The fixed tissue was dehydrated in a graded series of acetone to 70%, then treated with a saturated solution of uranyl acetate in 70% acetone for 24 hr at 4 C. Dehydration was continued in 100% acetone and tissues were infiltrated, under vacuum, with epoxy resin. Embedded tissue was sectioned on a Reichert OMU3 ultramicrotome, stained with lead citrate, and examined in a JEM 7 electron microscope.

Detection of geminiviral nucleic acids in leaves by squash and dot blot hybridization. Leaf disks were excised with a flame-sterilized No. 4 cork borer. For the squash blot procedure, leaf disks were squashed on Zetabind nylon membrane (CUNO, Inc., Meriden, CT) with a flame-sterilized round-bottomed glass rod. Two dot blot procedures were used, one in which one or three leaf disks were ground in 0.5 ml of distilled water or TE buffer (10 mM Tris, pH 7.5, 1 mM EDTA) in 1.5-ml Eppendorf tubes with a sterilized plastic pestle, and 10-μl samples were spotted onto Zetabind. The other procedure involved grinding a single leaf disk in 50 μl of TE, making twofold serial dilutions of the resulting plant sap in microtiter plates (Becton Dickinson Co., Lincoln Park, NJ), and spotting 5-μl samples of each dilution on Zetabind (dilution method). Similar dilution series were prepared from sap of mock-inoculated plants and from a solution containing 2 ng/μl of cloned BGMV-GA components A and B, and 5-μl samples were spotted on Zetabind.

Membranes were placed on filter paper, which had been saturated with 0.2 N NaOH, for 5 min to lyse cells and denature DNA, and then washed for 5 min in 1 M Tris, pH 7.5, then 2× SSC (23), followed by 95% ethanol. Membranes were air-dried and stored or used immediately for hybridization. Prehybridization (10 min) and hybridization (16–24 hr) were carried out in a solution of 30 (low stringency) to 50% (high stringency) formamide-0.125 M Na_2HPO_4-7% SDS-1 mM EDTA (pH 8.0) at 42 C. Membranes were washed twice at 42 (low stringency) or 65 C (high stringency) for 20 min with 0.125 M Na_2HPO_4-2% SDS-1 mM EDTA (pH 8.0), and once at the same temperature for 20 min in 20 mM Na_2HPO_4-1% SDS-1 mM EDTA (pH 8.0) (1). Membranes were blotted dry and exposed at −70 C to Kodak X-Omat AR or OG-1 X-ray film with Cronex Lightning Plus or Lanex intensifying screens, respectively. Exposure time varied from 4 to 24 hr.

DNA probes. Full-length DNA clones of BGMV-BZ, BGMV-GA, BGMV-DR, and BDMV-CO components A and B (15,16; R. L. Gilbertson et al, *unpublished data*) were used as DNA probes. Probe DNA was radioisotope-labeled with [^{32}P]-dATP by nick translation (23) with kits from Bethesda Research Laboratories (Gaithersburg, MD) or Promega BioTech (Madison, WI).

RESULTS

Sap transmission. Three separate experiments were conducted in Brazil to determine if BGMV-BZ could be sap-transmitted. Inoculum was collected from beans with golden mosaic symptoms in the field at CNPAF (field inoculum) or from beans infected with

Table 1. Results of experiments on the sap transmission of a Brazilian isolate of bean golden mosaic (BGMV-BZ) geminivirus

	Plants with symptoms/total plants[a]	
Treatment	Greenhouse	Growth chamber
Field inoculum[b]	0/30	0/48
Greenhouse inoculum[c]	0/127	0/200
Control-buffer plus Carborundum	0/74	0/85
Control exposed to viruliferous whiteflies	60/60[d]	...

[a] Plants with any symptoms of viral infection were counted. Numbers represent totals of three separate experiments.

[b] Sap prepared from leaves collected from bean plants with golden mosaic symptoms in the field.

[c] Sap prepared from bean leaves infected with BGMV-BZ by whiteflies in the greenhouse and included separate inoculations with sap from leaves collected 10, 17, or 21 days after exposure to viruliferous whiteflies.

[d] All 60 plants had golden mosaic symptoms. Large nuclear inclusion bodies were observed in phloem parenchyma cells of leaves from 10 randomly selected plants. Strong hybridization signals were observed from squash and dot blots of leaves from 10 plants after being probed with ^{32}P-labeled BGMV-BZ DNA.

BGMV in a greenhouse at 10, 17, or 21 days after exposure to viruliferous whiteflies (greenhouse inoculum). We were unable to transmit BGMV via plant sap, regardless of the inoculum source, whereas beans exposed to viruliferous whiteflies developed golden mosaic symptoms 7–10 days after exposure (Table 1).

Light and electron microscopy of bean leaves having viral symptoms in Brazil. Trifoliolate leaves from beans with golden mosaic symptoms were collected from the field and greenhouse (plants infected via whiteflies) at three stages of growth: very young, just beginning to expand; one-half to three-quarter expanded and showing mild (vein clearing) or severe symptoms (brilliant golden mosaic); or fully expanded leaves with golden mosaic symptoms. Leaves were also collected from healthy plants.

After azure-A treatment, blue-stained nuclear inclusions were observed in phloem parenchyma cells of bean leaves with BGM (Fig. 1A) but not in healthy leaves. Inclusions were most readily observed in one-half to three-quarter expanded trifoliolate leaves that showed vein clearing and were just beginning to develop golden mosaic symptoms. These leaves were usually collected from plants at the V3–R3 stages of growth (plants with first trifoliolate leaves to plants just after flowering) (22). Inclusions were not readily observed in very young leaves (less than one-half expanded), because of difficulties in visualizing nuclei, or in older fully expanded leaves where there was a scarcity of inclusions and nuclei were collapsed. Other nuclear abnormalities were observed only in leaves with BGM symptoms such as hypertrophy of nuclei and nucleoli and nucleolar elongation and vacuolization (Fig. 1A). Ultrastructural studies of leaves with BGM revealed hypertrophy of nuclei and nucleoli, nucleolar segregation, and the presence of fibrillar rings in nuclei of phloem parenchyma cells (Fig. 1B). These ultrastructural changes were not observed in nuclei of phloem parenchyma cells of healthy leaves.

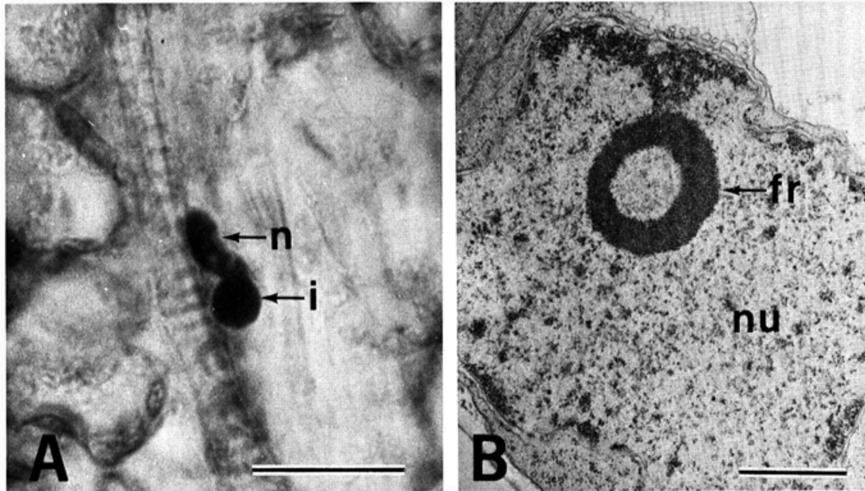

Fig. 1. (A) Light micrograph of infected bean leaf tissue stained with azure-A showing a nuclear inclusion body and nucleolar hypertrophy and vacuolization associated with infection by a Brazilian isolate of bean golden mosaic geminivirus. i = inclusion body, n = nucleolus. Bar = 10 μm. **(B)** Electron micrograph of a fibrillar ring observed in the nucleus of bean leaf tissue infected with a Brazilian isolate of bean golden mosaic geminivirus. fr = fibrillar ring, n = nucleus. Bar = 1 μm.

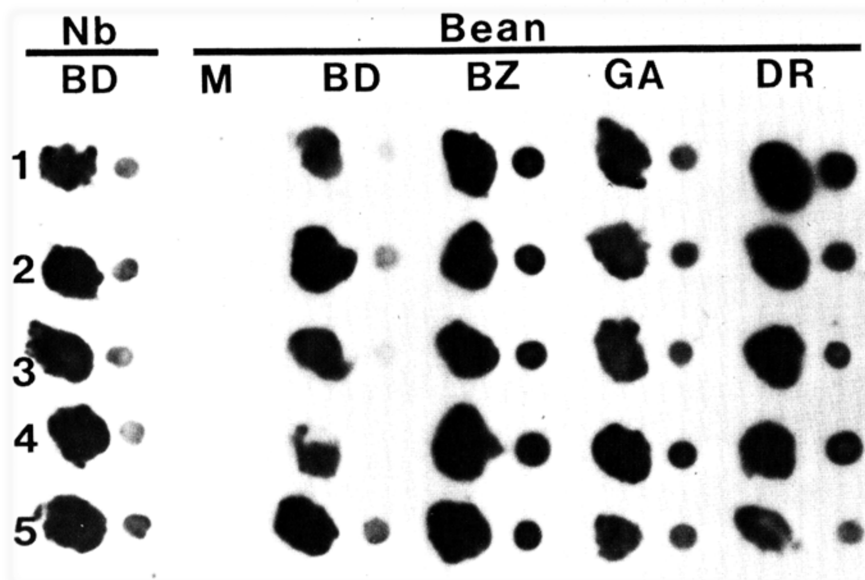

Fig. 2. Comparison of squash (samples on left in each column) and dot (samples on right in each column) blot hybridization for detection of bean-infecting geminiviruses in *Nicotiana benthamiana* (Nb) and bean (*Phaseolus vulgaris*) leaf tissue. Samples are mock-inoculated beans (M), plants infected with bean dwarf mosaic geminivirus (BD), or beans infected with a bean golden mosaic geminivirus (BGMV) isolate from Brazil (BZ), Guatemala (GA), or the Dominican Republic (DR). Samples were taken from each of five plants (1–5) for each treatment. Samples were hybridized with a general probe, which was a mixture of the cloned DNA components A and B of BGMV-BZ, BGMV-GA, and BDMV, using low-stringency conditions.

Sap transmission of BGMV-GA, BGMV-DR, and BDMV to beans and *N. benthamiana*. Bean (cv. Topcrop) and *N. benthamiana* plants were inoculated with BGMV-GA, BGMV-DR, and BDMV in Madison, WI. Five plants were inoculated with each isolate and the experiment was repeated three times. All of the bean plants inoculated with BGMV-GA and BGMV-DR developed golden mosaic symptoms and 14 of 15 bean plants inoculated with BDMV were dwarfed. *N. benthamiana* plants inoculated with BGMV-GA and BGMV-DR did not develop symptoms, whereas 13 of 15 plants inoculated with BDMV were stunted and leaves were cupped, distorted, and had mosaic symptoms. These results confirm the sap transmission of these isolates to beans (25,26) and indicate that BDMV can infect *N. benthamiana*.

Detection of geminiviral DNA in bean and *N. benthamiana* leaves by nucleic acid squash and/or dot blot hybridization with general and specific DNA probes. Dot and squash blots of leaves infected with BGMV-GA, BGMV-DR, BGMV-BZ, and BDMV were hybridized under low-stringency conditions with a general probe, which was a mixture containing cloned DNA components A and B of BGMV-BZ, BDMV, and BGMV-GA or BGMV-DR, or hybridized under high-stringency conditions with specific probes, which were the individual component B clones of these virus isolates. For BGMV-BZ, leaves from beans infected via whiteflies were collected at CNPAF, frozen or dried, and brought to Madison. Subsequently, we successfully infected beans with a mixture of the cloned DNA components A and B of BGMV-BZ (14); and in some cases, leaves from these infected plants were used for detection of BGMV-BZ. Before preparation of dot and squash blots,

dried leaf samples were rehydrated with sterile distilled water for 10–15 min, whereas frozen leaf samples were thawed for 1–5 min.

With the general probe, geminiviral nucleic acids were detected in squash and dot blots of bean leaves infected with BGMV-BZ, BGMV-GA, BGMV-DR, and BDMV and in *N. benthamiana* plants inoculated with BDMV (Fig. 2), but not in mock-inoculated plants (Fig. 2) or *N. benthamiana* inoculated with BGMV-GA or BGMV-DR (*data not shown*). When dot and squash blots of these infected leaves were probed with the specific probes, the BGMV-BZ component B probe hybridized only with tissue infected with BGMV-BZ (Fig. 3), the BDMV component B probe hybridized only with tissue infected with BDMV, and the BGMV-GA and BGMV-DR component B probes each hybridized only with tissue infected with BGMV-GA-and BGMV-DR (*data not shown*). For the general and specific probes, signals from infected tissues could be visualized after 4 hr of exposure, with very strong signals observed after 12–16 hr. These experiments were repeated three times, with similar results obtained for each experiment.

We now routinely use squash blot hybridization to confirm results of sap-transmission experiments, results of experiments involving inoculation of plants with cloned geminiviral DNAs, and to detect geminiviral nucleic acids in bean samples from Central America, South America, and the Caribbean. In all cases (more than 300 samples have been tested), hybridization signals have been observed in squash blots from beans with typical golden mosaic symptoms (no false negatives), and no signals have been observed in squash blots from beans without symptoms (no false positives).

The stability of squash and dot blotted samples on membranes before lysis was evaluated by preparing membranes with squash and dot blot samples from uninfected bean trifoliolate leaves and leaves infected with BGMV-GA. One membrane was exposed to lysis buffer immediately, one after a 2-wk storage, and the other after a 2-mo storage at 22 C. Membranes were probed with BGMV-GA component A 24–48 hr after exposure to lysis buffer. This experiment was conducted twice. We observed no reduction in hybridization signal 2 mo after samples had been applied to membranes. After exposure to lysis buffer, geminiviral nucleic acids can be detected on membranes for at least 1 yr and probably longer.

To determine if we could detect differences in viral nucleic acid titer in bean leaves by dot blot hybridization, beans (cv. Topcrop) were inoculated with BGMV-GA or BDMV on three separate dates (2–3 wk apart) so that infected trifoliolate leaves at different stages of development could be sampled. Infected leaves were collected from plants at three stages of development: V2 growth stage (22), young, partially expanded (7–10 days after inoculation); R1 growth stage, medium-aged, fully expanded (21–28 days after inoculation); and R6 growth stage, old, fully expanded, and beginning to senesce (49–56 days after inoculation). In a separate experiment, young, medium-aged, and old trifoliolate leaves infected with BGMV-GA or BDMV were collected from each of three bean plants 42 days after inoculation (R2 growth stage). Leaf disks were cut from leaves, dot blotted (dilution method), and membranes were probed with BGMV-GA or BDMV DNA components A and B under high-stringency conditions. These experiments were repeated three times. For both BGMV-GA and BDMV, the strongest hybridization signals were always observed from young partially expanded trifoliolate leaves, regardless of whether the leaves were collected from plants 10 or 42 days after inoculation. Less intense signals were observed from fully expanded trifoliolates, and the weakest signals were from older fully expanded trifoliolates that were beginning to senesce (*data not shown*).

Dot blot hybridization was also used

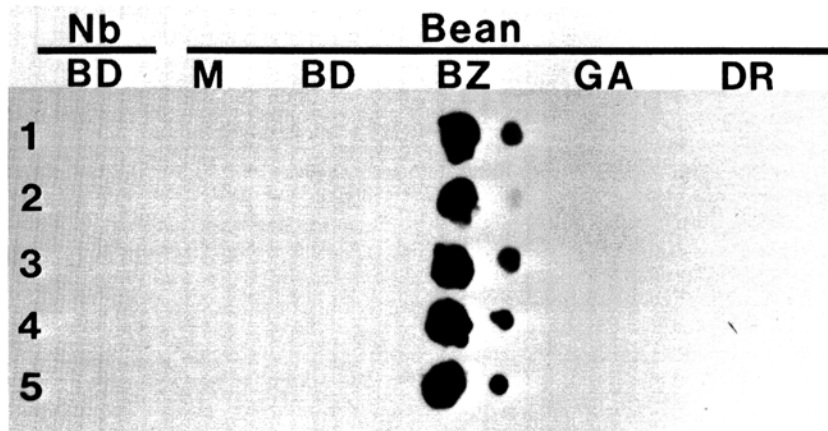

Fig. 3. Specific detection of a bean golden mosaic geminiviral isolate from Brazil (BGMV-BZ) in infected bean leaves by nucleic acid squash (samples on left) and dot blot (samples on right) hybridization. Samples are mock-inoculated leaves (M), *Nicotiana benthamiana* (Nb), or beans (*Phaseolus vulgaris*) infected with bean dwarf mosaic geminivirus (BD), or beans infected with BGMV-BZ or BGMV isolates from Guatemala (GA) or the Dominican Republic (DR). Samples were taken from each of five plants (1–5) for each treatment and were hybridized with the cloned DNA component B of BGMV-BZ using high-stringency conditions.

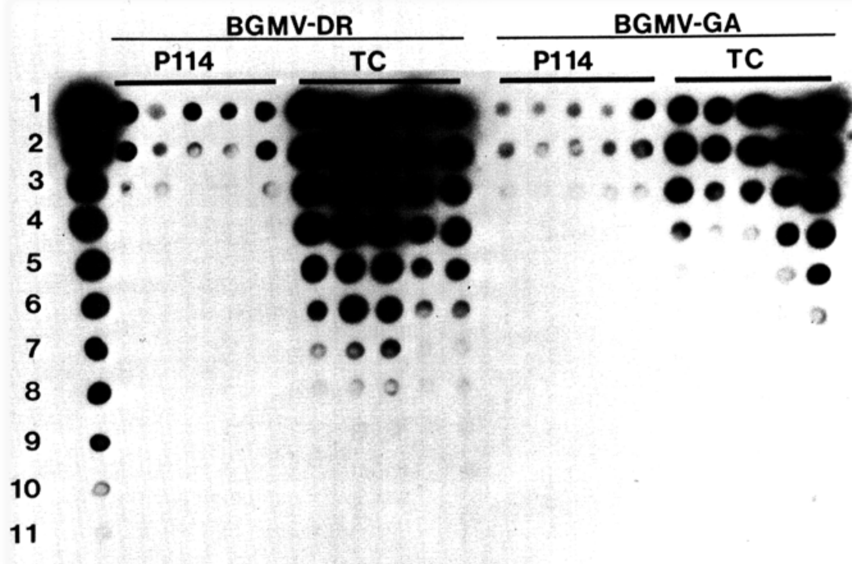

Fig. 4. Detection of differences in bean golden mosaic geminiviral nucleic acid titer in a moderately resistant bean cultivar, Pinto 114 (P114), and a susceptible cultivar, Topcrop (TC), by nucleic acid dot blot hybridization. Samples were prepared from infected first trifoliolate leaves from each of five individual plants of each cultivar inoculated with a BGMV isolate from Guatemala (BGMV-GA) or the Dominican Republic (BGMV-DR). Column one contains twofold dilutions (1–11) of cloned DNA components A and B of BGMV-GA (10 ng, 5 ng, 2.5 ng, 1.3 ng, 625 pg, 313 pg, 156 pg, 78 pg, 39 pg, 20 pg, 10 pg). Samples were hybridized with the cloned DNA components A and B of BGMV-GA using high-stringency conditions.

to detect differences in BGMV-GA and BGMV-DR nucleic acid titers in a susceptible cultivar (Topcrop) and in two cultivars that are moderately resistant or tolerant (HMX 7958 and Pinto 114). HMX 7958 is an experimental cultivar that has been reported to be moderately resistant (reduced symptoms) to BGM in Mexico and was kindly provided by the Harris Moran Seed Co. (San Juan Bautista, CA). Pinto 114 was reported to develop only moderate golden mosaic symptoms under field conditions, and when sap-inoculated with a BGMV-GA isolate, some plants did not develop symptoms (25). These cultivars were planted in a controlled environment chamber, and 7 days later, when beans had one-half expanded primary leaves, plants were inoculated with BGMV-GA or BGMV-DR (only Pinto 114). Ten to 14 days later, the first trifoliolate leaves with symptoms were collected from five infected plants of each cultivar and disks cut from these leaves were analyzed by dot blotting (dilution method). Membranes were probed with BGMV-GA components A and B under high-stringency conditions. The experiment with HMX 7958 was repeated three times, and the experiment with Pinto 114 was conducted twice. HMX 7958 plants developed mild golden mosaic symptoms after inoculation with BGMV-GA but not the brilliant golden mosaic, leaf distortion, and poor pod set that was observed with susceptible cv. Topcrop. Similarly, Pinto 114 developed golden mosaic symptoms after inoculation with BGMV-GA or BGMV-DR that were less severe than those observed on Topcrop. Greater hybridization signals were observed from dot blots of the susceptible Topcrop than from cvs. HMX 7958 (*data not shown*) or Pinto 114 (Fig. 4). Stronger hybridization signals also were observed from Topcrop plants infected with BGMV-DR than from those infected with BGMV-GA (Fig. 4). By making serial dilutions of plant sap and dotting samples of each dilution, the differences in viral nucleic acid titer could be clearly seen; in both HMX 7958 and Pinto 114, viral nucleic acids were not detected after two to three dilutions, whereas viral nucleic acids were detected in Topcrop after five to eight dilutions. These results clearly demonstrate that, at least for the infected first trifoliolate leaves, HMX 7958 and Pinto 114 had lower viral nucleic acid titers than Topcrop.

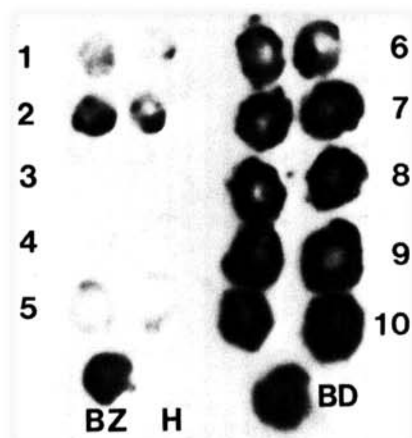

Fig. 5. Detection of geminiviral nucleic acids in bean and weed samples collected in the Dominican Republic by nucleic acid squash blot hybridization. Samples are **(1,2)** *Rhynchosia minima* with golden mosaic symptoms, **(3,4)** *Sida* spp. with golden mosaic symptoms, **(5)** *Jatropha* spp. with golden mosaic symptoms, and **(6–10)** beans with golden mosaic symptoms. H = bean plant with no symptoms, BZ = bean plant infected with a bean golden mosaic geminiviral isolate (BGMV) from Brazil, BD = bean plant infected with a bean dwarf mosaic geminiviral (BDMV) isolate from Colombia. Samples 1–10 were applied as duplicate samples. Samples were hybridized with a mixture of cloned DNA components A and B of BGMV isolates from Brazil and the Dominican Republic and BDMV.

Survey of beans and weeds with golden mosaic symptoms in the Dominican Republic. A trip was taken to the Dominican Republic in February 1990 to prepare squash blots of beans and weeds with golden mosaic symptoms for hybridization with the general or specific geminiviral DNA probes. Beans with golden mosaic, stunting, leaf distortion, and/or green mosaic symptoms; various crops with or without viral symptoms; or weeds with or without golden mosaic symptoms were collected from 24 locations in the Central and Western Dominican Republic. Each membrane had a sample from a bean plant with no symptoms (negative control) and a bean plant with typical BGM symptoms (positive control). The samples were squash blotted on four replicate membranes for hybridization with the general and specific probes. Before samples were lysed and hybridized in Madison, WI, samples from plants infected with BDMV and BGMV-BZ were squash blotted onto each of the replicate membranes to provide positive controls for the specific probes.

When the general probe was used, hybridization signals were not observed from crop samples (tomato, cassava, green pepper, or tobacco), weeds without golden mosaic symptoms, or beans without symptoms (Fig. 5); hybridization signals were observed from all beans with golden mosaic symptoms (Fig. 5), six of seven beans with stunting and leaf distortion, and one of four beans with green mosaic (similar to bean common mosaic symptoms). Weeds with golden mosaic symptoms that gave weak to strong hybridization signals with the general probe included: *Croton lobatus* L., *Jatropha* spp. (Fig. 5), *Sida* spp., *Urena lobata* L., *Bastardia bivalvis* (Cav.) Kunth., *Rhynchosia minima* (L.) Dc. (Fig. 5), and *Euphorbia heterophylla* L. No hybridization signals were observed from five of nine *Sida* spp. (see Fig. 5 for an example of *Sida* spp. without signal) and two of four *E. heterophylla* plants with golden mosaic symptoms.

With the BGMV-DR-specific probe at high-stringency conditions, hybridization signals were observed from beans with golden mosaic symptoms from all locations, whereas no signals were observed with the BDMV or BGMV-BZ specific probes (*data not shown*). Three bean plants with stunting and/or crinkling symptoms that hybridized with the general probe also hybridized with the specific probes; two of these did not hybridize with any of the specific probes, whereas one hybridized with the BDMV-specific probe. The only weed that hybridized with the specific probes was *R. minima*, which gave strong hybridization signals with all three specific probes.

DISCUSSION

Whitefly transmission, symptomatology, and light and electron microscopy were used to confirm that a geminivirus (BGMV-BZ) was associated with bean golden mosaic at CNPAF. In contrast to BGMV isolates from Central America and the Caribbean, BGMV-BZ could not be sap-transmitted to beans despite the use of a procedure successfully used for transmission of BGMV isolates from Florida and Puerto Rico (E. Hiebert, *personal communication*). Moreover, BGMV-GA, BGMV-DR, and BDMV-CO were easily transmitted with sap prepared by simply grinding infected leaves in 0.1 M potassium phosphate buffer. The apparent lack of sap transmissibility of BGMV-BZ is consistent with results of other researchers (7,11). Attempts to sap transmit BGMV from Argentina have also been unsuccessful (F. Morales, *unpublished data*). These results indicate that isolates of BGMV from Brazil and those from Central America and the Caribbean may be genetically different.

Thus, there is a need to determine the distribution of various bean-infecting geminiviruses throughout Latin America and to develop techniques for their rapid identification in beans and other plants, particularly weeds. Detection of inclusion bodies by light microscopy confirmed that a geminivirus was associated with BGM in Brazil, and the cytopathology of these leaves was similar to that for other geminiviral infections (6,12,20). However, using viral inclusion bodies for diagnosis is time consuming, technically difficult, and is generally diagnostic only for a viral group and not for virus strains (5,6).

We have demonstrated that DNA probes can rapidly detect and differentiate geminiviruses that infect bean. Squash blot hybridization with cloned geminiviral DNA was a rapid, efficient,

and reliable method for detecting all four geminiviral isolates used in this study. For example, we used squash blots to confirm that BDMV infected *N. benthamiana* and that BGMV-GA and BGMV-DR did not infect this host.

By using excised leaf disks, many samples can be squash blotted on a single membrane. The procedure is very flexible—fresh, frozen, or dried tissue can be used, and once tissues are squashed on membranes, hybridizations can be done months later. Thus, samples can be squashed at different locations and times, and lysis and hybridizations can be conducted at a later date at a central location. Similar results were reported when squash blot hybridization was used to detect tomato yellow leaf curl geminivirus (27).

The dot blot method was used to detect geminiviral nucleic acids in plant tissues and differences in geminiviral nucleic acid titers. The highest geminiviral nucleic acid titers were always detected in young expanding trifoliolate leaves. This observation agrees with results of an earlier study using physical measurements of geminiviral concentrations (31). Thus, young trifoliolate leaves should be collected for detection of geminiviral nucleic acids rather than fully expanded leaves.

It was also possible to detect differences in viral nucleic acid titers among bean cultivars with dot blot hybridization. It has been suggested that resistant cultivars may have lower viral titers than susceptible cultivars (3,13), and the dot blot assay showed that the viral nucleic acid titers in two moderately resistant cultivars (HMX 7958 and Pinto 114) were considerably lower than the titer in the susceptible cv. Topcrop. These results indicate that some viral function (e.g., replication or spread) may be inhibited in HMX 7958 and Pinto 114, and they support previous observations that these cultivars are moderately resistant to BGMV (S. Magneson, *personal communication*, 25). Dot blot hybridization can provide only an initial indication as to whether a cultivar may be resistant, and field evaluations must follow.

By using individual DNA components of these geminiviruses or mixtures of these components and varying the hybridization stringency, we developed a general probe that detected all four geminiviruses and specific probes that detected either BGMV-BZ, BDMV, or BGMV-GA and BGMV-DR. General and specific DNA probes have been used for detection and differentiation of barley yellow dwarf luteovirus serotypes (34). Polston et al (29) used a mixture of cloned DNA components as a general probe to detect two strains of squash leaf curl geminivirus and unique restriction fragments of the components of these strains as specific probes to differentiate the strains. For the general probe used in this study, a mixture of DNA-A and DNA-B components of BGMV-BZ, BDMV, and BGMV-GA or BGMV-DR was used under low-stringency conditions. The DNA-A component was included because of the presence of the highly conserved coat protein gene of the geminiviruses transmitted by whiteflies (27). This general probe should detect other geminiviruses transmitted by whiteflies. For example, this general probe detected geminiviral nucleic acids in tomatoes from Florida that showed symptoms of a geminivirus infection in November 1989 (R. L. Gilbertson et al, *unpublished data*). Conversely, because of the significant level of DNA sequence divergence that exists among the DNA-B components of BGMV-BZ, BDMV, and the closely related geminivirus isolates, BGMV-GA and BGMV-DR (15,16, R. L. Gilbertson et al, *unpublished data*), the cloned DNA-B components of these isolates can be used as specific DNA probes when used under high-stringency conditions.

We used the squash blot hybridization method and the general and specific probes to conduct a survey of potential weed hosts of BGMV and to evaluate the genetic diversity among BGMV isolates in the Dominican Republic. Our results clearly showed that the predominant BGMV isolate in the Dominican Rebublic was similar to BGMV-DR and that viruses related to BGMV-BZ and BDMV were not present in beans with golden mosaic symptoms. Thus, BGMV-DR could be used for screening bean lines for BGM resistance in the Dominican Republic. One stunted bean with crinkled leaves was possibly infected with BDMV or a related geminivirus, whereas two other plants with these symptoms were infected with a different and unknown geminivirus. Because beans with these symptoms were infrequently encountered, these other geminiviruses do not appear to be important in the Dominican Republic. Although numerous weeds had golden mosaic symptoms and geminiviral nucleic acids were detected in some of these, it appears that most of these weeds were not infected with BGMV-DR, BGMV-GA, BDMV, or BGMV-BZ, because they hybridized only with the general probe. The leguminous weed *R. minima*, which hybridized with all three specific probes, may be infected with more than one geminivirus or with a geminivirus that is closely related to those used in this study.

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