Generation and Characterization of Three Monoclonal Antibodies Useful in Detecting and Distinguishing Bean Golden Mosaic Virus Isolates

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ABSTRACT

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Two monoclonal antibodies prepared to purified virions of bean golden mosaic virus (BGMV) isolates from Guatemala (GA) and the Dominican Republic (DR) and one monoclonal antibody prepared to the coat protein of a BGMV isolate from Brazil (BZ) expressed in *Escherichia coli* were useful serological probes for detection of whitefly-transmitted geminiviruses. Abutilon mosaic, BGMV isolates from Puerto Rico (PR), and Homestead, Florida (H), BGMV-BZ, -DR, and -GA isolates, euphorbia mosaic, rhynchosia mosaic, squash leaf curl, soybean yellow mosaic, an isolate from *Macroptilium lathyroides* in Florida, and tomato mottle geminiviruses all reacted efficiently in Western blot and enzyme-linked immunosorbent assay with monoclonal antibody 3F7, indicating that this monoclonal antibody has a broad spectrum of reactivity. Another monoclonal antibody (2G5) reacted only with BGMV-DR, -GA, and -PR isolates. A third monoclonal antibody (5C5) reacted only with BGMV-BZ. A new geminivirus infecting cabbage (Brassicaceae) in Florida did not react with any of the three monoclonal antibodies.

Additional keywords: geminivirus coat protein sequences.

Bean golden mosaic geminivirus (BGMV) is one of the most devastating viral pathogens of beans in Latin America, particularly in Argentina, Brazil, Central America, and the Caribbean region, and recently in Florida. The geminivirus symptoms in infected bean plants, which may vary according to the geographical origin of the isolates, are characterized by striking yellow-green mosaic and by stunted and distorted plant growth (17). In addition to the biological variations among viruses described as BGMV, some have also been shown to have quite distinct genomic sequences (7). This genetic variation among BGMV isolates has been exploited to generate general and specific hybridization probes for the detection and differentiation of BGMV isolates (10). However, the appropriate technology for application of hybridization probes is not readily available in some of the countries affected by BGMV. In addition, the outcome of hybridization results with closely related genomic sequences may be subject to conditions of hybridization. Thomas et al (22) demonstrated that monoclonal antibody (MAb) probes could be used to distinguish closely related strains of the African cassava mosaic geminivirus (ACMV). Givord et al (11) selected two MAbs prepared against particles of a Nigerian isolate of ACMV that differentiated African geminiviruses (tomato yellow leaf curl, TYLCV; ACMV from West and East Africa; and okra leaf curl virus) from those found in other areas of the world (euphorbia mosaic virus; Indian cassava mosaic virus; and TYLCV India), in a double antibody sandwich enzyme-linked immunosorbent assay (ELISA). Swanson et al (21) studied the relationships among 14 isolates of whitefly-transmitted geminiviruses from the Americas and two from other continents using 27 MAbs prepared to ACMV and Indian cassava mosaic virus (ICMV). The serological reactivities of these MAbs ranged from two ACMV MAbs that reacted with all the American isolates to six MAbs that reacted with only one or two of them. Fifteen ACMV or ICMV MAbs did not react with any of the 14 American isolates. Pinner and Markham (18) resolved four strains of maize streak geminivirus (maize, sugarcane, panicum, and *Digitaria setigera*) among 19 isolates by ELISA using polyclonal antisera cross-absorbed with particles of the maize strain. The ability to identify these strains/variants in field samples by serology opens alternatives for rapid diagnosis of specific geminivirus infections.

We have screened a number of MAbs prepared to BGMV isolates from Guatemala (BGMV-GA), the Dominican Republic (BGMV-DR) and Brazil (BGMV-BZ). Here we show that these serological probes can be used to identify and distinguish BGMV isolates and other whitefly-transmitted geminiviruses. A preliminary report of this research has been presented (5).

MATERIAL AND METHODS

Virus cultures. The BGMV-DR and BGMV-GA isolates used in this study were purified (17) and freeze-dried in Cali, Colombia. The viruses were resuspended with water for use as immuno-

gens. Freeze-dried tissue, from Colombia, also was used as inoculum for the maintenance of these viruses in common bean (*Phaseolus vulgaris* L. 'Top Crop'). Cultures of these isolates as well as BGMV-BZ and BGMV-PR (from Puerto Rico) were maintained under restricted conditions as outlined in a USDA importation permit #911885. Other geminiviruses tested include a BGMV isolate from Homestead, Florida (BGMV-H) (4), a geminivirus isolate from *Macroptilium lathyroides* in Florida (MaGV-FL; previously named BGMV-F) (13) tomato mottle virus (TMoV) (3,19), and a new geminivirus infecting cabbage (CGV) (2). Dried cultures of the geminiviruses abutilon mosaic virus (AbMV), squash leaf curl virus (SqLCV) from California, rhynchosia mosaic virus (RMV) from Puerto Rico, euphorbia mosaic virus (EMV) from Puerto Rico and an uncharacterized geminivirus from Colombia known as soybean yellow mosaic (GV-soybean) were also used.

Expression of the BGMV-BZ coat protein gene in bacteria. The coat protein gene (AV1) was amplified by polymerase chain reaction (PCR) from the viral DNA using primers (5' GGG GAA TTC ATG CCT AAG CGT GAT 3' [viral sense] and 5' GGG GGA TCC AAG CTT CTG GGA CGA 3' [complementary sense]) based on the sequence provided by D. Maxwell (9). The amplified BGMV-BZ coat protein gene, which included 168 nucleotides downstream from the stop codon, was purified by agarose gel electrophoresis, digested with *Eco*RI and *Bam*HI, and subcloned into the *Eco*RI/*Bgl*II sites of the pEth-3c expression vector (16). The pEth expression vector cloning site is downstream but in the open reading frame of the coding sequence for 20 amino-acid residues from the bacteriophage T7 gene 10. Protein expression from this vector generated a fusion protein containing the 20 amino acid residues of the bacteriophage T7 gene 10 at the N' terminus of BGMV-BZ coat protein.

The *E. coli* BL21 culture containing the constructed expression vector was incubated until the cell concentration reached 0.4–0.6 A_{660}. Protein expression was induced by the addition of β-D-isopropyl-thiogalactopyranoside (IPTG) (1 mM final concentration) and the culture was further incubated for 3–4 h. The expressed protein was purified by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and then electroeluted from the polyacrylamide gel. The eluted protein was dialyzed against water to remove free SDS before use as an immunogen.

Immunization with BGMV-DR and -GA virus preparations. Purified BGMV-DR and -GA virions were mixed (1:1) to obtain 50-μg samples for immunization. Four 7-wk-old BALB/c mice were used from the Hybridoma Core, Laboratory of the Interdisciplinary Center for Biotechnology Research at the University of Florida. The mice were each injected subcutaneously with 50 μg/200 μl purified virus mixed preparation emulsified 1:1 with Freund's complete adjuvant. Two booster injections (50 μg/200 μl, 1:1 with Ribi adjuvant intraperitoneally) were done at week 2 and week 4 with respect to the first injection. A bleeding was done after the third injection. The titer of polyclonal antibodies for all four mice was determined by using ELISA. The mouse that had the highest antibody titer was given a final boost (25 μg/200 μl 1:1 Ribi adjuvant, intraperitoneally).

Fusions for BGMV-DR and -GA antibody expressing cells. Three days after the last boost and 8 wk after the initial immunization, the mouse was sacrificed, the spleen was removed aseptically, and approximately 1.7×10^7 spleen cells were fused with approximately 2.3×10^6 Hla Sp2o myeloma cells in 1 ml of 50% polyethylene glycol (PEG) (14). Three 96-well microtiter plates were seeded with an average of approximately 3×10^5 cells per well.

Immunizations with expressed BGMV-BZ coat protein. Three female, 8-wk-old BALB/c mice were each immunized twice subcutaneously with 50 μg of purified BGMV-BZ coat protein (expressed in *E. coli*) /200 μl Ribi adjuvant. The third immunization with 75 μg protein/200 μl Ribi adjuvant per mouse was performed intraperitoneally. The immunized mice were bled twice. The test bleeding done after the third immunization showed a high antibody titer.

Cell fusions for the production of BGMV-BZ MAbs. One mouse that showed the highest titer of antibodies to BGMV-BZ in the test bleeding was selected for the standard fusion protocol (14). Spleen cells (approximately 6×10^6 cells per milliliter) were fused with myeloma cells (approximately 1×10^6 cells) in 1 ml of 50% PEG. One plate was seeded with an average of 1.9×10^5 cells per well. The resulting hybridomas in each well were screened 10 days later by ELISA and imunoblotting.

Immuno-screening of MAbs. Antibody-trapped and antigen-trapped ELISAs were used for screening. For antibody-trapped tests, the wells were coated (100 μl per well) with polyclonal antiserum #1110 prepared to purified MaGV-FL (13) at 1:1,000 dilution in coating buffer and incubated overnight at 4 C or for 1 h at 37 C. After incubation, the plates were washed 3–10 times with phosphate-buffered saline containing Tween 20 (PBS-T) (5 min per wash). The antigen samples were prepared by grinding test plant tissue in PBS with a mortar and pestle and squeezing the extract through prewetted miracloth or cheesecloth. The tissue was extracted at a 1:5 ratio of tissue to buffer (50 mM carbonate, pH 9.6) for plate-trapped ELISA, and at a 1:10 ratio of tissue to PBS, pH 7.4, for antibody-trapped ELISA. Plate wells were loaded with 100 μl of extract per well and plates were incubated at room temperature (RT) for 1 h. The wells were washed once with PBS-T and blocked with 1% bovine serum albumin (BSA) in PBS (300 μl per well) for 1 h. The plates were washed 3× as before. For plate-trapped ELISA, polystyrene ELISA plate wells (Dynatech Laboratories, Chantilly, VA) were coated with 1 μg/ml purified virus or tissue extracts from beans infected with BGMV-GA, BGMV-DR, or from noninoculated bean plants, and incubated overnight before use. The serological reactions were detected by routine ELISA protocols.

Hybridoma cells were screened after 10 days of culture in the selective media. Of 288 wells, 260 wells showed a cell culture ready for screening. Initial screening was done with antigen-trapped ELISA using wells coated with 1 μg/ml of purified virus mixture of BGMV-DR and -GA. Hybridoma culture supernatant fluids were used undiluted in the tests. Those hybridoma cells producing MAbs that were positive to BGMV-DR and -GA were retested with antibody- and plate-trapped ELISAs, against tissue extracts infected singly with BGMV-BZ, -DR, and -GA, and from noninoculated bean plants, in order to determine the MAb specificity. Purified mouse IgG at 1:100 dilution, preimmune polyclonal mouse serum, buffer, and conditioned media were used as controls.

Dot immunoblotting. A nitrocellulose membrane prewetted in 20 mM Tris buffered saline (TBS), pH 7.5, for 20 min was positioned on the slotted portion of the acrylic plate of a Surf-Blot 10 apparatus (Surf-Blot from Idea Scientific, Minneapolis, MN). The optimum sap dilution of tissue infected singly with BGMV-BZ, -DR, and -GA was previously determined to be 1:5. The tissue extracts, dissociated with 3% SDS and heated to 95 C for 2 min, were applied (150 μl per channel). The Surf-Blot was then incubated for 30 min at RT with agitation. The channels were washed twice with TBS. The apparatus was disassembled for the 90 degree rotation of the membrane. Twenty-eight supernatants from the hybridoma cell cultures that had tested positive for BGMV by ELISA were used full strength (150 μl per channel). The Surf-Blot was incubated for 30 min as before. The channels were washed and the Surf-Blot device was disassembled. The nitrocellulose membrane was washed briefly in TBS, then blocked with Blotto (TBS with 5% nonfat dry milk) containing 1% BSA. Goat antimouse IgG alkaline phosphatase-conjugate was applied at 1:1,000 dilution in Blotto as the second antibody for 1 h, washed and finally incubated in substrate (Nitro blue tetrazolium, chlo-

ride/5 bromo-4 chloro-3 indolyl phosphate p-toluidine salt) about 10 to 40 min.

Western blot tests. Extracts from geminivirus-infected tissues and noninfected tissues were subjected to SDS-PAGE (12% gel). The separated proteins were transferred to nitrocellulose membranes for immunoblot analysis, using a Bio-Rad Mini-PROTEAN II electrophoresis cell and Bio-Rad Trans-Blot electrophoretic transfer cell (Bio-Rad Laboratories, Richmond, CA). Nitrocellulose membranes were blocked overnight with Blotto plus 1% of noninfected tissue extracts. The blots were incubated with the MAbs at 1:2,000 in Blotto. After three successive washings in TBS-T, blots were incubated with alkaline phosphatase-conjugated goat antimouse IgG diluted 1:1,000. Before the addition of substrate solution (as above for dot immunoblotting) the blots were washed 4 times. The results were determined qualitatively according to development of purple color.

Preparation of bean tissue infected with BGMV-BZ. In order to screen the clones resulting from the cell fusions for the production of BGMV-BZ MAbs, it was considered necessary to have fresh, BGMV-BZ–infected tissue. Geminivirus-infected tissue extracts stored frozen showed reduced activity in ELISA (data not presented). BGMV-BZ is sap transmissible only at very low levels by mechanical transmission. We obtained infectious clones of the BGMV-BZ (9) from D. Maxwell (University of Wisconsin). The cloned DNA was amplified in *E. coli* and young bean seedlings were inoculated with the DNA by biolistic bombardment, using a helium gas-driven system developed by Gray et al (12). About 10–25% of the bombarded seedlings developed BGMV-BZ symptoms. Tissue extracts from infected leaves were used to screen the fusion clones for reactivity with BGMV-BZ and to identify MAb lines producing antibody specific for the virus.

Ascites production. Selected cell lines were recloned twice by limiting dilution and the subclones were tested for constancy in reactivity and specificity. The clones with the highest reading in ELISA were selected for ascites production in pristane-primed BALB/c mice by injecting hybridoma cells into the peritoneal cavity.

TABLE 1. Reactivity (A_{405}) of monoclonal antibodies 3F7 and 2G5 to geminiviruses in indirect enzyme-linked immunosorbent assay[a]

Type of test and antigens	3F7	2G5
Antibody-trapped[b]		
BGMV-DR[c]	2.464[d]	2.466
BGMV-H	2.555	0.107
TMoV (tomato)	1.273	0.061
TMoV (tobacco)	0.504	0.062
Bean (healthy)	0.077	0.063
Tomato (healthy)	0.069	0.056
Plate-trapped[e]		
BGMV-DR	1.078	0.150
BGMV-H	1.282	0.066
TMoV (tomato)	0.256	0.063
TMoV (tobacco)	0.154	0.069
Bean (healthy)	0.088	0.066
Tomato (healthy)	0.106	0.071

[a] Tests done in Bradenton, FL.

[b] For antibody-trapped tests, wells were coated with a polyclonal antiserum #1110 prepared to MaGV-FL at a dilution of 1:1,000 and incubated at 4 C overnight. The antigen (tissue extracts diluted 1:10 in phosphate-buffered saline [PBS]) was incubated for 1 h at room temperature (RT). The wells were blocked for 1 h at RT with PBS–bovine serum albumin (1%) after being washed 10× with PBS-T. Primary monoclonal antibody diluted 1:5,000 was added and incubated for 1h at RT. The secondary antibody, goat antimouse antibody conjugated to alkaline phosphatase, diluted 1:2,000, was added and incubated for 1 h at RT. The wells were incubated with substrate 1/2 h prior to reading.

[c] BGMV-DR, BGMV isolate from Dominican Republic; BGMV-H, a BGMV isolate from Homestead, FL; TMoV, tomato mottle virus.

[d] Values are the average of triplicate readings.

[e] For plate-trapped tests, wells were coated with antigen diluted 1:5 in coating buffer and incubated for 1 h at RT. The rest of the conditions were as indicated in footnote b above.

TABLE 2. Reactivity (A_{405}) of monoclonal antibodies 3F7, 2G5, and 5C5 to geminiviruses in indirect enzyme-linked immunosorbent assay[a]

Antigens	3F7	2G5	5C5
Bean, healthy[b]	0.03[c]	0.01	0.00
BGMV-GA	2.58	2.63	0.05
BGMV-DR	2.64	2.61	0.05
BGMV-BZ	2.59	0.14	2.26
BGMV-PR	2.59	2.59	0.10
BGMV-H	2.64	0.28	0.05
MaGV-FL	2.68	0.29	0.03
BDMV	2.53	0.11	0.06
TMoV	0.48	0.06	0.05
Tomato, healthy	0.03	0.07	0.07

[a] Tests were done at CIAT, Cali, Colombia. Wells were coated with a polyclonal antiserum #1110 prepared to MaGV-FL at a dilution of 1:800 and incubated at 4 C overnight. The antigen (tissue extracts diluted 1:10 in phosphate-buffered saline [PBS]) was incubated for 1 h at room temperature (RT). The wells were blocked for 1 h at RT with PBS–bovine serum albumin (1%). Monoclonal antibody diluted at 1:8,000 was added and incubated for 1 h at RT. Goat antimouse antibody conjugated to alkaline phosphatase, diluted 1:2,000 was added and incubated for 1 h at RT. The wells were incubated with substrate for 1/2 h prior to reading.

[b] BGMV-BZ, BGMV isolate from Brazil; BGMV-GA, BGMV isolate from Guatemala; BGMV-PR, BGMV isolate from Puerto Rico; MaGV-FL, a geminivirus isolated from *Macroptilium lathyroides* weed in Florida; BDMV, bean dwarf mosaic virus. Other abbreviations as in Table 1.

[c] Values are the average of four separate well readings.

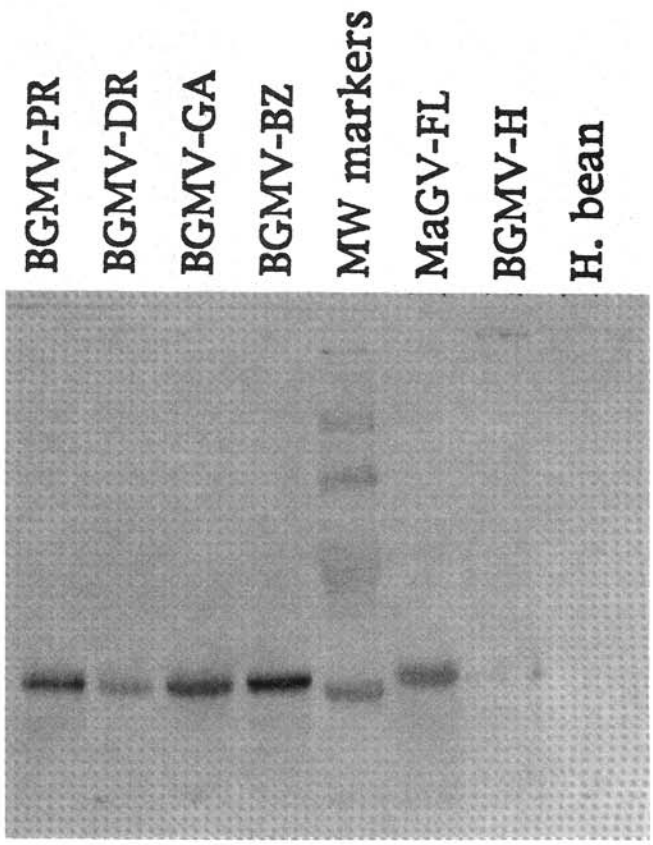


Fig. 1. Western blot analysis with the monoclonal antibody 3F7 prepared to BGMV-DR and -GA. Lanes from left to right are: geminiviruses are as listed in Tables 1 and 2 and molecular weight (MW) markers and extracts from healthy bean (H. bean) are in the lanes indicated. MW markers were prestained proteins with estimated molecular weights of 215K, 105K, 70K, 43K, 28.5K, 18K, and 15.4K, obtained from GibcoBRL. The position of the 28.5K marker protein is slightly below the positions of the geminivirus coat proteins on the membrane.

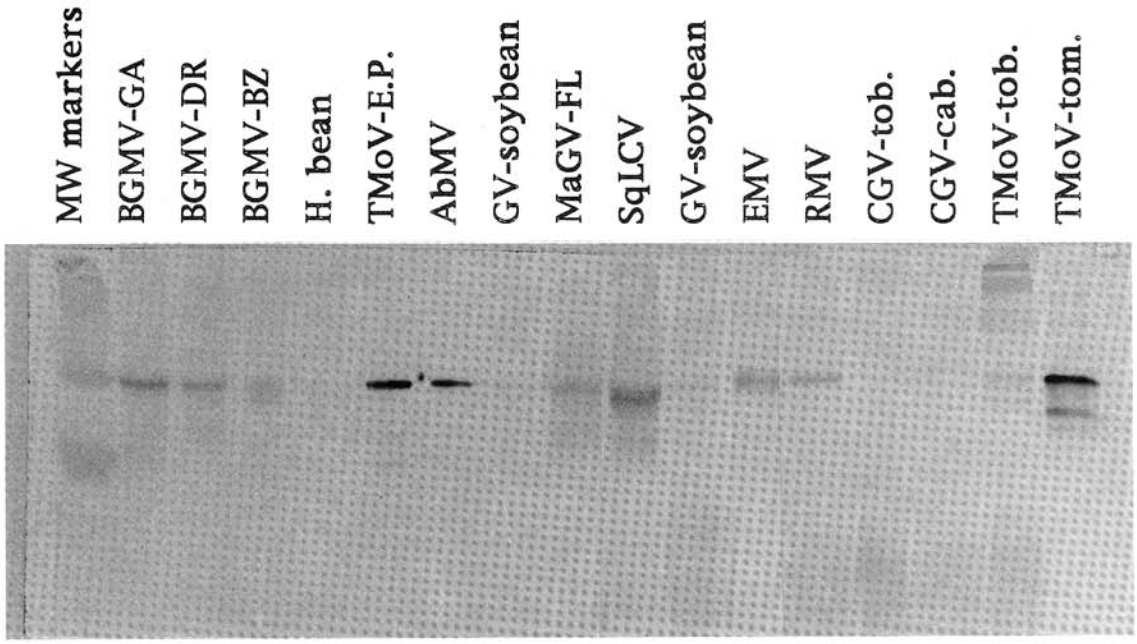


Fig. 2. Western blot analysis with the monoclonal antibody 3F7 prepared to BGMV-DR and -GA, tested against various geminiviruses. Lanes are: MW molecular weight markers, H. bean = extracts from healthy bean, and geminiviruses are as listed in Tables 1 and 2 and in Materials and Methods.

RESULTS

Immunoscreening for BGMV-DR and -GA MAbs. The ELISA screening of the culture media from the cells in (260) individual microwells (derived from the hybridoma cells after fusions with the BGMV-DR and -GA sensitized mouse spleens) indicated that 25% of the cell lines reacted with a mixture of purified virions from BGMV-DR and -GA isolates. Sixty-eight positive cell lines were selected for further screening with ELISA (plate- and antibody-trapped) against these two BGMV isolates, BGMV-BZ, and noninfected tissue extracts in order to determine specificity. Of the 28 cell lines selected from the 68 positive wells on the basis of high titer and virus specificity, 20 reacted with tissue extracts from all three BGMV infections and 8 reacted only with BGMV-DR and -GA.

Twelve of the 28 ELISA-positive cell lines were also positive in the dot immunoblot assay with BGMV-DR and -GA. Additional geminiviruses, TMoV and MaGV-FL, were included in these tests. Three of the 12 cell lines reacted specifically with the BGMV-DR and -GA isolates (narrow specificity), whereas the other 9 cell lines reacted with all the geminiviruses tested (broad specificity). A MAb line (designated 3F7) from the broad spectrum reactivity group and one (designated 2G5) from the narrow specificity group were selected for ascites production after two limiting dilution clonings for the selection of a single cell line.

Immunoscreening of the BGMV-BZ MAbs. Antibody-trapped and plate-trapped ELISA were performed for cell line immunoscreening. The first screening was done with purified, expressed coat protein of BGMV-BZ. Immunoblot screening was performed with previously prepared membranes (expressed coat protein) with the same hybridomas tested by ELISA. Twenty-six supernatants (of all the fusions from the 96 well plate) tested positively in ELISA to *E. coli*–expressed BGMV-BZ coat protein, and 24 of these 26 supernatants were also positive by immunoblot analysis. A second screening was performed to determine serological specificity. ELISA plates were coated with tissue extracts from plants infected with BGMV (-BZ, -DR, and -GA), MaGV-FL, noninfected host plants, with TMoV (1) coat protein expressed in *E. coli*, and *E. coli* suspensions (without coat protein expression). The MAb line designated as 5C5 was the only one from the 24 positive cell lines found to be specific to BGMV-BZ.

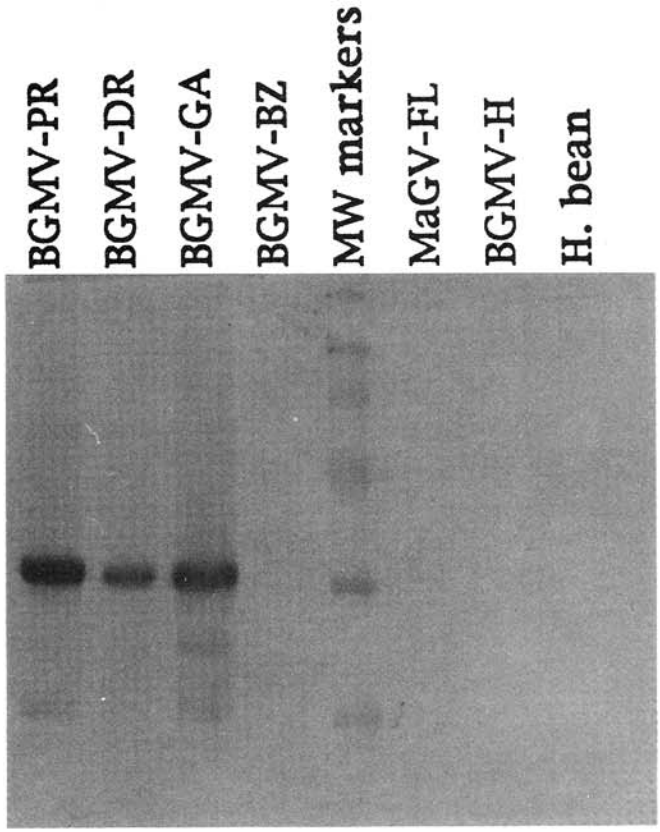


Fig. 3. Western blot analysis with the monoclonal antibody 2G5 prepared to BGMV-DR and -GA. Lanes from left to right are: geminiviruses are as listed in Tables 1 and 2 and molecular weight (MW) markers and extracts from healthy bean (H. bean) are in the lanes indicated.

Serological reactivity of the selected MAbs. The MAb 3F7 was found to react with all the BGMV isolates and with seven other whitefly-transmitted geminiviruses, but not with CGV (Tables 1 and 2; Figs. 1 and 2). This MAb 3F7 was designated a broad spectrum serological probe for whitefly-transmitted geminiviruses. A more specific MAb (2G5) was identified that reacted

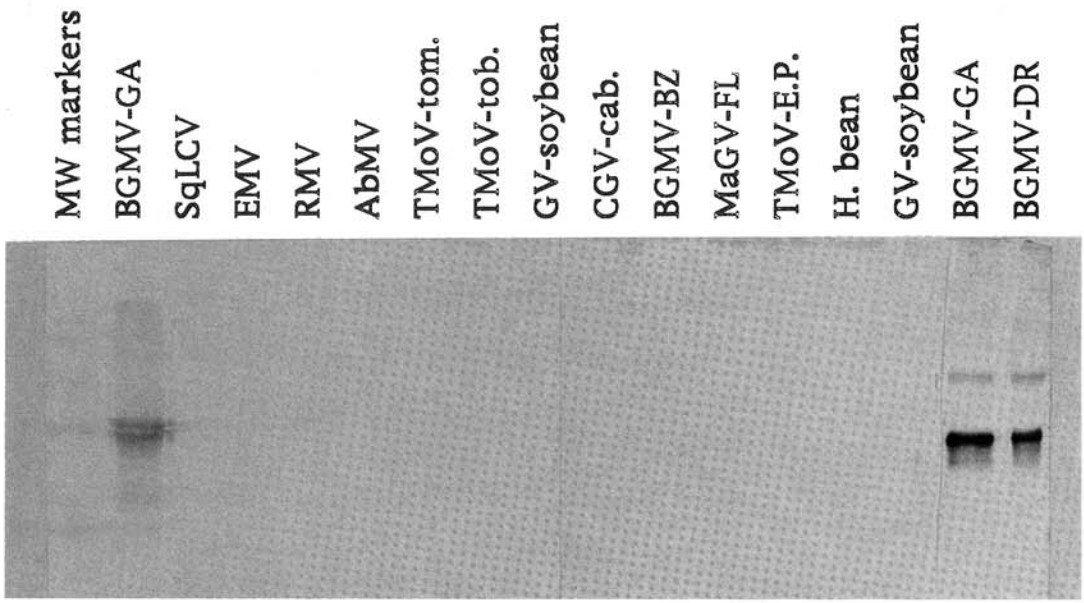


Fig. 4. Western blot analysis with the monoclonal antibody 2G5 prepared to BGMV-DR and -GA, tested against various geminiviruses. Lanes are: MW molecular weight markers, H. bean = extracts from healthy bean, and geminiviruses are as listed in Tables 1 and 2 and in Materials and Methods.

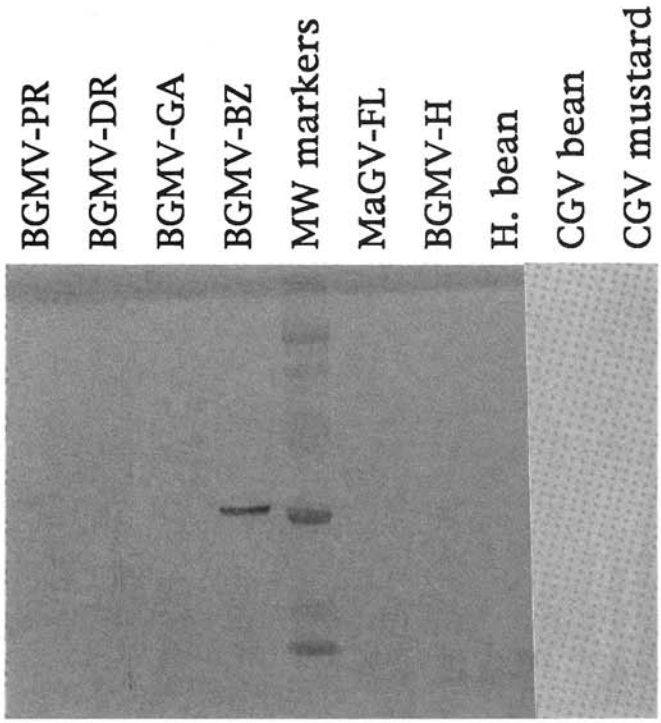


Fig. 5. Western blot analysis with the monoclonal antibody 5C5 prepared to BGMV-BZ. Lanes from left to right are: geminiviruses are as listed in Tables 1 and 2 and in Materials and Methods. Molecular weight (MW) markers and extracts from healthy bean (H. bean) are in the lanes indicated.

with BGMV isolates from the Dominican Republic, Guatemala, and Puerto Rico but not with BGMV isolates from Florida and Brazil, MaGV-FL, or with the other geminiviruses (Tables 1 and 2; Figs. 3 and 4). A third MAb (5C5) was identified that only reacted with BGMV-BZ (Table 2 and Fig. 5). For ELISA tests, the best results were obtained when polyclonal antibody to MaGV-FL was used to trap antigen prior to testing with the MAbs (Table 1). The selected MAbs were also reactive in Western blots (Figs. 1–5) as well as in ELISA. The weak reactions, observed for the GV-soybean, SqLCV, RMV, EMV, and AbMV in Figure 4, may have been due to the use of extracts from dried tissue samples for these viruses. Fresh tissue samples were not available.

The electrophoretic mobility of the coat protein in SDS-PAGE for all the tested geminiviruses was similar (Figs. 1–5). The estimated coat protein size of 30 kDa is close to that calculated from the predicted amino acid sequences (Fig. 6).

DISCUSSION

Several MAbs reactive with the BGMV isolates from the Dominican Republic and Guatemala were selected; however, no MAbs were identified that distinguished these two isolates. Sequence information (7) for the two isolates, BGMV-DR and -GA, indicates that the predicted coat protein amino acid sequences are nearly identical (they differ in three amino acid residues near the amino-terminus, positions 10, 11, 15, and one at position 90; Fig. 6). The generation of a MAb (3F7) reactive to a wide range of geminiviruses is not unexpected because of the high degree of amino acid residue homology among coat proteins of whitefly-transmitted geminiviruses (Fig. 6) (20). The identification of a MAb that is specific for BGMV-GA, -DR, and -PR but that does not react to closely related coat proteins of other geminiviruses indicates that MAbs can distinguish closely related antigens. MAbs having similar broad spectrum and narrow spectrum specification for ACMV isolates were identified from those generated for a ACMV isolate in a study by Thomas et al (22). Swanson et al (21), in a survey of 14 geminiviruses from the Americas with a panel of MAbs to ACMV and ICMV, also found a range of broad to very narrow spectra of serological reactivities with certain MAbs.

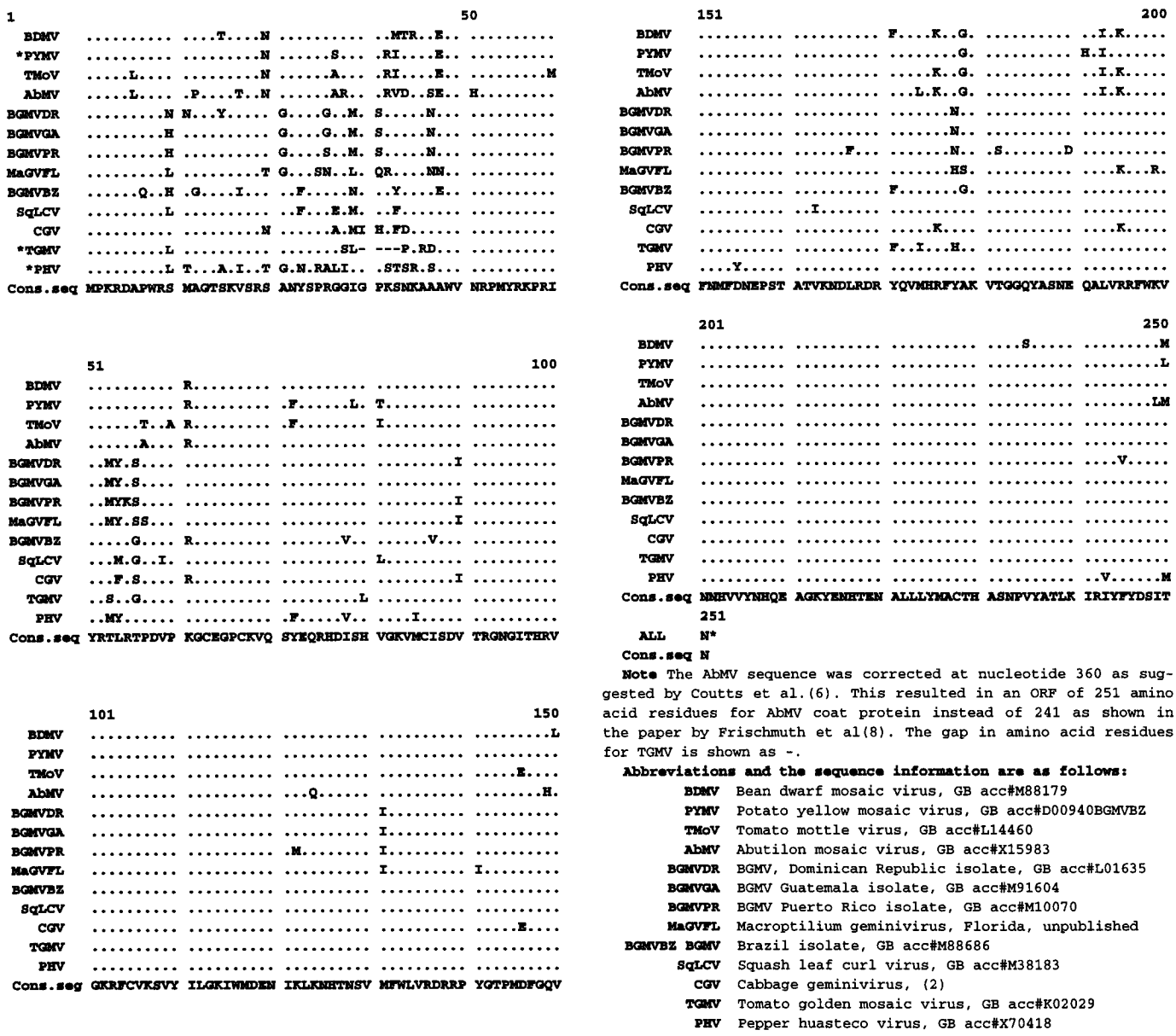
Although the secondary structure, antigenicity, and hydrophilicity profiles of the predicted amino acid sequences of the coat proteins have not been analyzed, an examination of the amino acid sequences of a number of whitefly-transmitted geminiviruses (Fig. 6) reveals some interesting differences with possible relevance to their reactivities to the three MAbs. For example, the BGMV isolates -DR, -GA, and -PR, the only three isolates that react with MAb 2G5, share unique amino acid residues at positions 31 (serine), and 178 (asparagine) of their coat proteins compared with those of other whitefly-transmitted geminiviruses shown in Figure 6. There are other amino acid differences at the primary structure level among the coat proteins that may be im-

portant in identifying the epitopes responsible for the specificities of the three MAbs. The significance of these observations, however, must be verified by site-directed mutagenesis studies.

The development of a MAb line producing antibody specific for BGMV-BZ using an immunogen obtained by expressing the BGMV-BZ coat protein gene in *E. coli* demonstrates the usefulness of this approach for the development of serological probes. We also have applied this technology to develop (low background), high titer polyclonal antibodies to BGMV-BZ, TMoV, and CGV (A. M. Abouzid, E. Hiebert, and D. E. Purcifull, *unpublished*). Expressing geminivirus coat proteins in *E. coli* circumvents the difficulties involved in purifying geminivirus virions in sufficient amounts for use as immunogens and provides coat protein free from serologically reactive plant host constituents. The denaturation of the *E. coli*–expressed BGMV-BZ coat protein during purification by SDS-PAGE did not prevent the generation of high affinity MAb antibodies reactive with BGMV-BZ in ELISA tests.

The selected MAb cell line 3F7 (used for ascites production) is a very useful broad spectrum serological probe for whitefly-transmitted geminiviruses, although it failed to react with CGV. This MAb has a high affinity for its epitope as indicated by its reactivity at dilutions of up to 1:32,000 in ELISA and 1:8,000 in Western blots. The MAb 3F7 has been used in the routine identification of TMoV in field tomato samples in Bradenton, FL (J. E. Polston, *unpublished data*) and in Leesburg, FL (S. A. Webb, *personal communication*) in ELISA tests. Two MAbs developed to ACMV by Givord et al (11) were found useful in screening cassava plants in field tests for the presence of ACMV, in detecting whitefly-transmitted geminiviruses from various plants and from various geographical origins, in assessing virus concentrations in plants, and in searching for natural reservoirs of the viruses. The selected MAb cell line 2G5 is a specific serological probe for BGMV-DR or -GA or -PR. MAb 2G5 also does not react with a sida-type geminivirus (1) recently found infecting beans in Florida (A. M. Abouzid and E. Hiebert, *unpublished*). This MAb also has a high affinity for its epitope and works well at high dilutions in ELISA and Western blots. The MAb 5C5 selected for the BGMV-BZ is specific for BGMV-BZ, has a high affinity for its epitope, and it is reactive in both ELISA and immunoblot tests.

In comparative tests done at CIAT the MAbs were more dis-



Note The AbMV sequence was corrected at nucleotide 360 as suggested by Coutts et al.(6). This resulted in an ORF of 251 amino acid residues for AbMV coat protein instead of 241 as shown in the paper by Frischmuth et al(8). The gap in amino acid residues for TGMV is shown as -.

Abbreviations and the sequence information are as follows:

BDMV	Bean dwarf mosaic virus, GB acc#M88179
PYMV	Potato yellow mosaic virus, GB acc#D00940BGMVBZ
TMoV	Tomato mottle virus, GB acc#L14460
AbMV	Abutilon mosaic virus, GB acc#X15983
BGMVDR	BGMV, Dominican Republic isolate, GB acc#L01635
BGMVGA	BGMV Guatemala isolate, GB acc#M91604
BGMVPR	BGMV Puerto Rico isolate, GB acc#M10070
MaGVFL	Macroptilium geminivirus, Florida, unpublished
BGMVBZ BGMV	Brazil isolate, GB acc#M88686
SqLCV	Squash leaf curl virus, GB acc#M38183
CGV	Cabbage geminivirus, (2)
TGMV	Tomato golden mosaic virus, GB acc#K02029
PHV	Pepper huasteco virus, GB acc#X70418

Fig. 6. A comparison of the amino acid residues of the coat proteins of some of the geminiviruses tested by the MAbs. Geminiviruses marked by * have not been tested with the MAbs and are shown for comparative purposes. Dots indicate amino acids that are the same as the consensus sequence.

criminating for the Latin American BGMV isolates than were the available nucleic acid hybridization probes (M. Cancino and F. Morales, *unpublished*). The MAb 2G5 did not react with BGMV-H (Fig. 4, lane 7), whereas nucleic acid hybridization probes prepared to the intergenic regions for the A and B component DNAs for BGMV-H failed to distinguish between BGMV-H and BGMV-GA under high stringency hybridization tests (4). Similarly, Swanson et al (21) show that anti-ACMV MAb SCR 19 distinguished between SqLCV isolates from Mexico and Arizona whereas the SqLCV DNA probes (representing the complete sequence; 15) failed to detect a difference. The information reported herein and the reports by Swanson et al (21), Pinner and Markham (18), Givord et al (11), and Thomas et al (22) show that geminivirus antibodies can be useful in identifying and distinguishing closely related geminiviruses.

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