Characteristics of a Latent Potyvirus Seedborne in Guar and of Guar Green-Sterile Virus

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ABSTRACT

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A symptomless, seedborne potyvirus was isolated from guar (*Cyamopsis tetragonoloba*) germ plasm in Griffin, Georgia. The host range and serology were similar to those reported for guar green-sterile virus (GGSV) and guar symptomless virus. Biological, serological, and molecular comparisons of the Georgia isolate and the South African GGSV indicate they are similar and are closely related to bean common mosaic potyvirus (BCMV). The Georgia isolate is seed-transmitted at a rate of up to 94% in guar line PI 340385. Sequence analysis of the capsid protein (CP) gene and the 3'-untranslated region (3'-UTR) showed that both isolates are 96% homologous. GenBank searches indicate that both are related to various strains of BCMV. The highest CP nucleotide sequence and 3'-UTR identities of 91 and 93%, respectively, were with those of BCMV-NL4. On this basis, both isolates from guar should be considered as strains of BCMV.

Guar (*Cyamopsis tetragonoloba* (L.) Taubert) is native to tropical Africa and Asia. Guar gum has been used as an emulsifier of salts in bakery and baking mixes; a cereal stabilizer; a cheese, dairy, and frozen dessert stabilizer; a firming agent in fats and oil; a thickener in gravies and sauces; and a stabilizer in jams and jellies. Guar is also used in industrial applications such as oil drilling muds, textile printing, warp sizing, paper coating, and carpet printing. The world market for guar gum is estimated to be around 150,000 tons/year, 70% of which is produced by India and Pakistan. Consumption by the United States is estimated to be about 40,000 tons/year. The United States guar germ plasm collection is maintained at the Plant Genetic Resources Conservation Unit in Griffin, Georgia, and the individual lines must be grown in the field to regenerate the seed when viability drops or when reserves are inadequate. These regenera-

tion plots are monitored to determine the presence and identity of pathogens which might affect seed production or subsequent seed distribution. The guar lines in this study were donated from India to the National Plant Germplasm System in 1969 and were regenerated first in 1970 at Vernon, Texas. In 1995, a number of guar lines were planted for increase. Extracted sap from some of these plants, with and without symptoms, reacted with a general potyvirus monoclonal antibody. Many plants that tested positive for potyvirus infection had obvious mosaic symptoms and reacted with peanut mottle potyvirus (PeMoV) antiserum in double-antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA). However, some plants in seven lines also reacted with bean common mosaic potyvirus-blackeye cowpea mosaic strain (BCMV-BICM) antiserum. Tests on seeds from these plants indicated a high rate of seed transmission. The lack of obvious symptoms and the highly seedborne nature of this virus in guar prompted this study.

Edwardson (8) lists a number of potyviruses that infect guar upon mechanical inoculation, including PeMoV and BCMV. Zaumeyer and Goth (24) reported that the type strain of BCMV induces latent infection of guar. However, only two potyviruses have been reported as naturally infecting guar. These are guar symptomless virus (GSV; 11) and guar green-sterile virus (GGSV; 3,18). The host range and serology closely matched that of the virus found in Georgia. An isolate of GGSV from South Africa was used for compari-

son. The GSV also had some biological and serological features that were similar, but was not available.

Molecular data on potyviruses have shown that the 3'-terminal region can be used as a reliable criterion to distinguish among strains of potyviruses (23). The sequences of the capsid protein (CP) and the 3'-untranslated region (3'-UTR) are the most useful regions of the potyviral genomes in taxonomic analysis. Therefore, the 3'-terminal 1359 nucleotides of the two potyviruses seedborne in guar were cloned and sequenced to ascertain their taxonomic status on the basis of the sequence analyses of the CP and 3'-UTR.

MATERIALS AND METHODS

Sampling and seed transmission. Initial testing of leaf samples from all symptomatic guar plants in field regeneration plots was by direct antigen coating (DAC)-ELISA with a monoclonal general potyvirus antiserum. Plants testing positive were then tested by DAS-ELISA with BCMV-BICM and PeMoV antiserum. Plants of guar plant introduction accessions (PIs) 338745, 340292, 340316, 340385, 340437, 340451, and 340588 showed slight to no vein clearing symptoms and tested positive with BCMV-BICM. Seeds from these virus-infected lines were grown in an insect-free greenhouse. The resulting seedlings were observed for symptoms and were tested by DAS-ELISA using BCMV-BICM antiserum after 4 weeks. Further testing of seed transmissibility was done using seeds collected from greenhouse-grown infected guar and with seeds from South Africa from GGSV-infected SL100 guar plants.

Host range. The following plants were grown in pots in an insect-free greenhouse and were inoculated with the guar virus isolates from Georgia (guar-US): *Arachis hypogaea* L. cv. Virginia Bunch (peanut); *Capsicum annuum* L. var. *annuum* (pepper); *Chenopodium album* subsp. *amaranticolor* Coste & A. Reynier; *Cucurbita pepo* L. cv. Early Crookneck (squash); *Lupinus albus* L. (white lupine); *Lycopersicon lycopersicum* (L.) Karsten var. *lycopersicum* (tomato); *Nicotiana benthamiana* Domin; *Nicotiana tabacum* L. cv. Burley 21 (tobacco); *Phaseolus vulgaris* L. cvs. Black Turtle Soup, Black Turtle II, Black Valentine, Bountiful, Butter VI, Cherokee, Cordon, Dubbele Witte, Great

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Northern II, Jamapa, Monroe, Orfeo, Pinto III, Plentiful, Stringless Green, Top Crop, and Winfield Pole (beans); *Pisum sativum* L. subsp. *sativum* cv. Little Marvel (pea); *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* cv. Coronet (cowpea); and PIs 471443, 471446, 471454, 471455, 471459, 471460, 471462, 471473, 471475, 471482, 471484, and 471485 (cowpeas). Comparative host range tests with the South African GGSV isolate included the bean and cowpea cultivars, *Chenopodium*, squash, pea, and *N. benthamiana* mentioned above. Inoculum for host range evaluations consisted of infected guar or Dubbele Witte bean tissue ground in 0.025 M phosphate buffer, pH 7.2, containing 1% celite. The inoculum was applied to leaves of healthy plants previously dusted with 600 grit Carborundum. All plants were young when inoculated: bean, cowpea, and white lupine plants in primary leaf stage; squash plants in cotyledon stage; *Nicotiana* and *Chenopodium* plants in three- to five-leaf stage; and pepper and tomato plants in three-leaf stage. Plants were grown in a greenhouse at 25 to 28°C under natural lighting in July and August. Symptoms were observed over a period of 3 weeks after inoculation. In addition, the guar-US isolate was compared with BCMV-US#5 (supplied as a gift by G. I. Mink, Prosser,

WA) on the bean lines listed above in a separate test. Inoculum for the US#5 virus came from infected Bountiful bean.

Antisera and ELISA. Polyclonal rabbit antiserum to bean yellow mosaic potyvirus (BYMV) PVAS-368 was supplied by the American Type Culture Collection; BCMV-BICM, BCMV-peanut stripe strain (PSt), and PeMoV antisera were from J. W. Demski, Griffin, Georgia; and cowpea aphid-borne potyvirus (CABMV) antiserum was provided by R. O. Hampton, Corvallis, Oregon. The general potyvirus monoclonal antibody (PTY-1) was obtained from Agdia Inc., Elkhart, Indiana. Antigens used as controls were as follows: BYMV (PV-368) was supplied by American Type Culture Collection; and BCMV-BICM, BCMV-PSt, and PeMoV were gifts from J. W. Demski.

For viral assays by DAS-ELISA, infected and control leaf tissue were triturated at a 1:9 dilution (wt/vol) in antigen buffer (9). Plates were coated with purified immunoglobulin G (IgG) and the antibody-conjugates were used at 1:500 for BCMV-BICM and at 1:750 for PeMoV.

For viral assays by DAC-ELISA, infected or control leaf tissue was triturated at 1:9 dilution (wt/vol) in extraction buffer (0.15 M NaCl, 0.036 M Na₂HPO₄, 0.016 M NaH₂PO₄, 0.013 M sodium dieth-

yldithiocarbamate, 0.003 M NaN₃, pH 7.1; 10) and further dilution was done in this buffer, if desired. These extracts were placed into duplicate wells per sample and incubated overnight at 4°C. Polyclonal rabbit antisera were diluted at the indicated rates (BCMV-BICM 1:18,000; BCMV-PSt 1:15,000; BYMV 1:10,000; CABMV 1:2,000) in healthy plant extract (1:50 wt/vol healthy cowpea tissue and conjugate buffer), pipetted into wells, and the plates incubated at 37°C for 1.5 h. Monoclonal antibody PTY-1 (1:200) was not cross-absorbed with healthy extract before use. Secondary antibody, goat anti-rabbit antibody or goat anti-mouse antibody, as appropriate, conjugated to alkaline phosphatase at 1:1,500 dilution, was added to plates and incubated 1.5 h at 37°C. After each step of incubations of plant extracts, of antisera, and of conjugates, plates were washed three times in phosphate buffered saline-Tween, pH 7.5.

After the final washing of the plates, p-nitrophenyl phosphate substrate was added and incubated at room temperature for 1 to 2 h. Absorbance (A₄₀₅) values of p-nitrophenol product were recorded on a Dynatech MR 700 plate reader (Dynatech Laboratories Inc., Chantilly, VA). Assays were considered positive when sample absorption values exceeded the mean value for healthy controls plus 2.5× the standard deviation among wells containing controls.

3' cloning and sequencing. Preparation of tissue extracts from infected leaves, Sephadex G-50 purification of total RNA preparations, and reverse transcription-polymerase chain reaction (RT-PCR) were as described previously (16). The oligonucleotide mixture 5'-dA₂₁(C/G/T)-3' used as downstream primer was as described (16). The upstream primer sequence was chosen from a conserved region (MIEAWG) in the N1b gene (17). This primer pair amplified part of the N1b coding sequence, the complete CP coding sequence, and the 3'-UTR. Primers were synthesized by the Molecular Genetics Instrumentation Facility at the University of Georgia, Athens. Standard recombinant DNA techniques were used (20).

PCR products were cloned into pGEM-T vector (Promega Corporation, Madison, WI) and the sequence was obtained by using an automated sequencer at the DNA Sequencing Core Facility of the Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville. Sequence analyses were done using Seqaid-II (19), University of Wisconsin Genetics Computer Group (UWGCG; 7). Multiple alignments were done using pileup available in the UWGCG package, and also by CLUSTAL-W (22). Phylogenetic trees were constructed using PHYLIP and unrooted trees were generated using TreeView (15). Sequence searches were done using BLAST (1) against those that were available in GenBank (4).

Table 1. Comparative host range for isolates of guar green-sterile virus from the United States (GGSV-US) and South Africa (GGSV-SA)

Test host plants ^a	GGSV-US	GGSV-SA
Dubbele Witte bean	Chlorosis, mild mottle, moderate leaf rolling, distortion of true leaves	Mottle, mild chlorosis, leaf rolling, distortion of true leaves
Black Turtle Soup bean	Moderate mottle, rugose, distortion of true leaves	Moderate mottle, rugose, distortion of true leaves
Black Turtle II bean	Moderate mottle, rugose, distortion of true leaves	Moderate mottle, rugose, distortion of true leaves
<i>Chenopodium amaranticolor</i>	Chlorotic local lesions with centers becoming red after 10 days	Chlorotic local lesions with some red centers after 10 days

^a The following plants were also included in this test, but had no symptoms and tested negative for virus by direct antigen coating-enzyme-linked immunosorbent assay: cowpea PIs 471462, 471482, 471454, 471443, 471473, and 471484; *Nicotiana benthamiana*; Little Marvel pea; Early Crook-neck squash. Three to eight plants of each test genotype were used and buffer-rubbed controls were run for each genotype.

Table 2. Serological comparison of isolates of guar green-sterile virus from the United States (GGSV-US) and South Africa (GGSV-SA) by direct antigen coating-enzyme-linked immunosorbent assay (DAC-ELISA)

Antigens ^b	Antisera ^a			
	BCMV-BICM	BCMV-PSt	CABMV	BYMV
GGSV-US 1/10	0.544	0.425	0.259	0.0
GGSV-US 1/50	0.497	0.193	0.0	0.0
GGSV-SA 1/10	0.566	0.488	0.338	0.0
GGSV-SA 1/50	0.289	0.383	0.072	0.0
BCMV-BICM 1/10	0.389	0.0	0.0	0.0
BCMV-PSt 1/10	0.527	0.282	0.0	0.0
BYMV 1/10	0.541	0.449	0.0	0.527

^a ELISA absorption (A₄₀₅) values (average reading for two wells each). BCMV-BICM = bean common mosaic potyvirus-blackeye cowpea mosaic strain; BCMV-PSt = BCMV-peanut stripe strain; CABMV = cowpea aphid-borne mosaic potyvirus; BYMV = bean yellow mosaic potyvirus.

^b Guar virus isolates from infected Dubbele Witte bean, BCMV-BICM from infected Early Ramshorn cowpea; BCMV-PSt from infected PI 306232 peanut; BYMV from infected Top Crop bean.

RESULTS

Seed transmission. At least one plant of each of seven guar lines in the regeneration plots reacted positively with the BCMV-BICM antiserum. Some of these infected plants failed to produce any harvestable seed, and many of the seeds produced were shriveled and failed to germinate (no data were taken on germinability of seeds at this stage of the experiment). Germinable seeds from those plants that did produce seeds were tested for seed transmission. Although there were only a few seeds, seed transmission was observed: PI 340385 had 29 positive seedlings of 51 total seedlings, PI 340588 had 7 of 9, PI 340437 had 4 of 65, and PI 340292 had 0 of 17. These infected seedlings were grown in the greenhouse, and the seeds were collected and stored at 5°C.

Seedlings of only PI 340385 produced viable seeds under these conditions; therefore, the following data deal solely with this line. As with field-produced seeds, greenhouse seed lots contained a number of shriveled seeds and had low germinability. One sample of 220 seeds yielded 65 seedlings that developed to a stage large enough to test. In a second sample of 225 seeds, 80 germinated, of which 60 survived to be tested. However, 94% of 261 seedlings that did survive in four tests were positive. In one test with seeds from GGSV-infected SL100 guar plants, 25 of the 44 surviving seedlings (57%) were positive by DAS-ELISA.

Host range. The host range tests were done initially with only guar-US isolate. It incited chlorotic local lesions on *C. amaranticolor* plants, which developed red centers as they aged. Of the many bean cultivars inoculated, plants of only Dubbele Witte, Black Turtle Soup, and Black Turtle 2 developed symptoms. On Dubbele Witte, there was chlorosis and mild mottling on the true leaves, accompanied by leaf rolling and distortion. When vining started, the upper leaves were chlorotic, distorted, and reduced in size. Both Black Turtle Soup and Black Turtle 2 had medium mottle symptoms on the true leaves, which were rugose and distorted. No symptoms were observed on the inoculated primary leaves of any bean cultivars. In comparison, BCMV-US#5 did not infect Dubbele Witte, but caused severe rugosity on true leaves of Black Turtle Soup. BCMV-US#5 also produced systemic infection on cvs. Cherokee, Bountiful, Black Valentine, Monroe, and Stringless Green. No virus was detected by ELISA in other bean cultivars or the other species. In independent inoculations of guar-US to the standard BCMV-host-range (14), no infections were detected (M. Silbernagel, *personal communication*). This suggests that the virus is in the BCMV subgroup, but is not BCMV.

The results from the comparative host range study of the two viruses shows that

they are similar based upon symptoms produced (Table 1).

Serology. Table 2 shows the results of DAC-ELISA with guar-US and GGSV with several BCMV antisera and with BYMV antiserum. These data also indicate a serological relationship of these two viruses with BCMV because of their relationship to BCMV-BICM, BCMV-PSt, and CABMV. The position of guar-US within the BCMV subgroup was confirmed by

tests (G. Mink, *personal communication*) against a collection of legume virus antibodies (13), and additional tests (R. Jordan, *personal communication*) against a panel of monoclonal antibodies reactive with various potyviruses (12).

3' cloning and sequencing. The nucleotide and the translated amino acid sequences of the 3' terminal regions of guar-US and GGSV are presented in Figure 1. Sequences have been submitted to Gen-

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ATGATCGAGGCATGGGGATATCCTGAGCTACTTCAAGAGATTAGGAAGTTTATCTGTGGCTACTTGAAA
M I E A G W Y P E L L Q E I R K F Y L W L L E R
GAGATGAGCTTAGGGAGATTGTCTGCTAGTGGAGGAGCTCCATATATAGCAGAGTCAGCGCTTAAACTCT
D E L R E I A A S G G A P Y I A E S A L K T L
TTACACGAACAAGAGAGCAAAGATTGAGGAGTTGGCAAATATCTGGAAGTCTCGATTTTAACTATGAG
Y T N K R A K I E E L A K Y L E V L D F N Y E
GTAGGATGCGGAGAATCTGTACACCTACAGTCAGGATCTGAACAACAACCACCATCAGTAGTGGACGCTG
V G C G E S V H L Q S G S E Q Q P P S V V D A G
GCGTGGATACTGGGAAGGACAAGAAGGATAAGGGTAGTAAAGGAAGACCCTGTGAGAAAGGATGGGCG
V D T G K D K K D K G S K G K D P V R K D G A
ACGACATAACAGTCGTGAACAGAGAGTTCAACAATGAGAGACAAGGACGTAGATGCAGGTTTCGAAAGGA
R H N S R E T E S S T M R D K D V D A G S K G
AAGATAGTTCCCGGCTTCAAAAGATCACAAAAGGATGAAGTGGCAATGGTAAAGGGGAATGTGATCT
K I V P R L Q K I T K R M N L P M V K G N V I L
TGAATTTAGATCATCTGTTAGATTACAAGCCAGAGCAAAGTATCTTCAACACAAGAGCAACAAAAT
N L D H L L D Y K P E Q T D L F N T R A T K M
GCAGTTTGAATGTGGTACAATGCTGTGAAGAGTGAAGTGAATAGATGATGACCAGATGGCAATTGTA
Q F E M W Y N A V K S E Y E I D D D Q M A I V
ATGAATGGCTTTATGTTGTTGTCATCGATAATGGCACCTCACCGGATGTGAATGGCACTTGGGTGATGA
M N G F M V W C I D N G T S P D V N G T W V M M
TGGATGGAGATGAACAAGTGGAGTACCCACTCAAACCAATGGTCGAGAATGCAAAACCAACACTTCGTCA
D G D E Q V E Y P L K P M V E N A K P T L R Q
AATTATGCACCATTTCTCAGATGCAGCTGAAGCATACATTGAGATGAGGAATTCGGAGAAACCGTATATG
I M H H F S D A A E A Y I E M R N S E K P Y M
CCTAGGTACGGACTTCTCCGAAATTTGAGGGATAAAAATCTAGCTCGTACGCTTTTGTATTCTATGAAG
P R Y G L L R N L R D K N L A R Y A F D F Y E V
TGACATCCAAAACATCGGATCGAGCGAGAGAAGCGGTAGCACAGATGAAGGCAGCAGCCCTCAGCAACGT
T S K T S D R A R E A V A Q M K A A A L S N V
TAGCAGCAAGTTGTTTGGACTTGTGTAATGTAGCAACAACAGCGAGAATACTGAAAGGCACACTGCA
S S K L F G L D G N V A T T S E N T E R H T A
AGAGACGTCAATCAGAATATGCACACACTTCTTGGCATGGGCCCTCCGAGTAAAGATTGGGTAAACTAA
R D V N Q N M H T L L G M G P P Q < 367
TCACAGTTAGCATCTCGCGTGAATAATTTTCATATAGTAGTCTTTTATGTTCTCTTTAGTTTCAGTG
TGGTTTCCACCACCTTTGTGTAATTTGTGATAGTGTGTTAGGCCACCAACATATTGTGGATACTTTA
TGTTTATGAGTAAAGCGGAAGAACCATTGCAATGGCGAGGGCATGCAGAGTATTTATCACGCCCGTG
AGGTAGCTACGGCAATGTTTGTGTTCCA 1359

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Fig. 1. Nucleotide (shown as DNA) and the deduced amino acid sequences of potyviruses seedborne in guar. Sequence of guar-US isolate is shown. Nucleotide and amino acid differences with respect to guar-US are indicated for the South African isolate of guar green-sterile virus (GGSV). Conserved amino acid sequences are underlined. Putative protease cleavage site between Nib and capsid protein is double underlined. Sequences have been submitted to Genbank as accession numbers AF045065 and AF045066 for guar-US and GGSV, respectively.

bank as accession number AF045065 and AF045066 for guar-US and GGSV, respectively. The cloned regions contained an open reading frame that codes for a polypeptide of 367 amino acids, followed by 255 nucleotides of untranslated region (Fig. 1). The protease cleavage site was presumed to be between glutamine and serine on the basis of the previously characterized cleavage sites for potyvirus CPs (Fig. 1). The surrounding peptide sequence also conformed to the established protease cleavage sequence context (21). The presence of the amino acid triplet DAG (Fig. 1) was consistent with aphid transmissibility of the virus (2). The other conserved regions along the capsid protein sequence are indicated in Figure 1. There was a high degree of nucleotide sequence identity (96%) in the region examined.

Based on sequence analysis, the CP was found to be 285 amino acid residues long. GenBank searches identified several BCMV strains (5) as the most closely related sequences to the guar isolates. Pairwise comparisons showed that guar-US and GGSV had the highest sequence identity to BCMV-NL4. Considering the large number of BCMV strains sequenced, and the high degree of sequence identity among them, only BCMV-NL4 (5) was compared with the guar-US and GGSV isolates. They were 91 and 89% identical, respectively, at the CP amino acid level. The CPs of guar-US and GGSV are the same length as those of BCMV-NL4 (Fig. 2A). Most of the sequence differences were located toward the amino terminal ends of the CPs, while the carboxy terminal half of the CPs were highly homologous to that of BCMV-NL4

(Fig. 2A). Phylogenetic analysis based on CP amino acid sequence comparison showed that guar-US and GGSV formed a tight cluster that was closest to the BCMV subgroup of potyviruses (Fig. 3).

The 3'-UTRs of guar-US and GGSV were 255 nucleotides long. Sequence relationships of the 3'-UTRs also showed that both had the highest sequence identity (93%) with that of BCMV-NL4 (Fig. 2B). Phylogenetic analysis using the 3'-UTR sequences also indicated that guar-US and GGSV were closer to the members of the BCMV subgroup than to any other known potyviruses (Fig. 4).

DISCUSSION

The reduction in seed quality and germinability without visible symptoms on the foliage of infected guar plants and the high rate of seed transmissibility associated with this viral disease make it of importance for germ plasm movement and quarantine procedures. The guar accession PI 340385 may be unusual, because it produces viable seeds when infected with this virus, and is certainly unusual with its 94% transmission rate. These rates are higher than those reported for GSV (12 to 28%), but this could be a genotypic difference in the guar lines (11).

The comparison of the host ranges of the guar-US and GGSV isolates under greenhouse conditions in Georgia indicate that they are very similar. The *C. amaranticolor* in our tests developed chlorotic local lesions with red centers which agrees with those reported for GSV (11); however, this plant was not studied in the tests on GGSV (3,18). The finding that the viruses cause similar symptoms on Dubbele Witte and Black Turtle Soup plants confirms the results of Rey et al. (18). There are a number of differences in the host range results obtained compared to those reported by Hansen and Lesemann (11) for GSV and Ben-Moshe et al. (3) and Rey et al. (18) for GGSV. Some of these differences can be attributed to differences in environmental conditions. Local reactions were observed on the inoculated leaves of Black Turtle Soup, Dubbele Witte, Monroe, and Top Crop plants in the South African studies (3,18) while our studies in Georgia failed to produce any such reactions. Rey et al. (18) reported a severe reaction of GGSV on *N. benthamiana* while we did not. This could be explained by a difference in genotypes of the species. The isolate of guar-US used for our host range studies was free of other viruses because it had been purified. This test produced results identical to those of the comparative test shown in Table 1. Ben-Moshe et al. (3) reported that GGSV caused symptoms on tomato and cowpea. This was not found in our experience or in that of Rey et al. (18).

Our serological results indicate a close relationship of the guar viruses to BCMV and some to CABMV, but none with

Fig. 2. (A) CLUSTAL-W generated multiple alignment of capsid protein sequences of bean common mosaic-NL4 (BCMV-NL4) and the two potyviruses seedborne in guar (guar-US and GGSV). (B) CLUSTAL-W generated multiple alignment of 3'-untranslated regions of BCMV-NL4 and guar-US and GGSV. Asterisks indicate identical residues and dots indicate chemically similar residues.

BYMV. Therefore, our serological evidence and that from South Africa (3,18) indicates that these viruses are in the BCMV subgroup.

Different potyviruses are reported to be 39 to 53% identical in the 3'-UTR region, while strains of the same virus exhibit identity of 83 to 99% among their UTR sequences (21,23). Similarly, the CP sequence identities are 38 to 71% among different viruses and 90 to 99% among virus strains (21,23). When compared to the known sequences of BCMV strains, identities between guar-US and GGSV ranged from 86 to 91%. These values are near the 90%-identity suggested by Shukla et al. (21) to be considered strains of a virus. Similar results were reported by Collmer et al. (6) for adzuki bean mosaic potyvirus (AzMV), wherein CP sequence identity between AzMV and the various BCMV strains fell short of the 90% value.

Our data also suggest that potyviral genomes exist as a continuum on an evolutionary scale and some viruses may constitute an exception to the proposed criteria for delineating viruses and strains. Nevertheless, on the basis of high degree of sequence identity and serological and biological characteristics, potyviruses guar-US and GGSV should be considered as strains of BCMV. We propose that the guar-US and GGSV be designated BCMV-GGS-US and BCMV-GGS-SA, respectively.

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Fig. 3. Cluster dendrogram showing the relationship among the capsid protein sequences of the two potyviruses seedborne in guar and those of other potyviruses. Sequences for comparison were obtained from GenBank (4). AzMV = adzuki bean mosaic, BCMNV = bean common mosaic necrosis, BCMV = bean common mosaic, BICMV = blackeye cowpea mosaic, CABMV = cowpea aphid-borne mosaic, DeMV = desmodium mosaic, GGSV = guar green-sterile, guar-US = isolated from guar in Georgia, PStV = peanut stripe, PWV = passion fruit woodiness, SAPV = South African passiflora, TEV = tobacco etch, and ZYMV = zucchini yellow mosaic potyviruses. TEV is used as an outgroup. Vertical lengths of the branches are arbitrary.

Fig. 4. Cluster dendrogram showing the relationship among the 3'-untranslated regions of the two potyviruses seedborne in guar and those of other potyviruses. Sequences for comparison were obtained from GenBank (4). AzMV = adzuki bean mosaic, BCMNV = bean common mosaic necrosis, BCMV = bean common mosaic, BICMV = blackeye cowpea mosaic, CABMV = cowpea aphid-borne mosaic, DeMV = desmodium mosaic, GGSV = guar green-sterile, guar-US = isolated from guar in Georgia, PStV = peanut stripe, PWV = passion fruit woodiness, SAPV = South African passiflora, TEV = tobacco etch, and ZYMV = zucchini yellow mosaic potyviruses. TEV is used as an outgroup. Vertical lengths of the branches are arbitrary.

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