Preliminary Characterization of a Potyvirus, the Causal Agent of Green-Sterile Disease of Guar

M. E. C. REY, A. SMITH, S. JOHNSON, and S. KHITSANE, Department of Microbiology, University of the Witwatersrand, Johannesburg, P O Wits, 2050

ABSTRACT

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A potyvirus named guar green-sterile virus (GGSV) has been reported to be associated with a disease of guar (*Cyamopsis tetragonoloba* (L.)) in southern Africa. Symptoms of leaf chlorosis/localized necrosis and sterility in guar were obtained using a sucrose-gradient purified GGSV preparation for mechanical inoculation. Potyviruses were also isolated from sterile guar plants grown from seed and from mechanically inoculated guar and bean cultivar hosts, which indicates that GGSV is the causal agent of disease symptoms in guar. Polyacrylamide gel electrophoresis, Western blot (immunoblot), serological analyses, and isolation of an RNA species of approximately 9.4 Kb indicate that GGSV is a potyvirus. Results of mechanical transmissions to bean cultivars used to differentiate bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) strains, comparisons with a local strain of BCMV (BCMV-SA), and serological tests with antisera to BCMV and BCMNV indicate that GGSV may serologically react with several BCMV strains but it is not a known strain or serotype of BCMV. Thus, GGSV may be a new guar legume potyvirus.

Sterility in guar (*Cyamopsis tetragonoloba* (L.) Taub.) has recently been reported in southern Africa (1). Affected guar plants exhibit fewer and often sterile inflorescences. The sterile plants sometimes have elongated stems and petioles that remain green long after healthy plants have senesced. Fifty percent of the seeds from infected pods are necrotic, shriveled, and discolored, and do not germinate readily, while healthy-appearing seeds from infected pods germinate and have no symptomatic leaf appearance. Affected guar leaves often exhibit chlorosis (Fig. 1A). This disease has been named green-sterile disease, and a potyvirus, guar green-sterile virus (GGSV), has been associated with the disease (1). Mechanical transmission to several *Phaseolus vulgaris* L. cultivars, with a crude infected-leaf preparation, resulted in localized red vein necrosis and chlorosis on primary inoculated leaves, but no systemic spread was previously observed (1). Inoculated guar leaves developed localized necrosis but no further sterile symptoms were discernible (1). Antisera to GGSV and bean common mosaic virus (BCMV) react with infected guar plants and mechanically inoculated beans, indicating that green-sterile symptoms observed in the field may be associated with infection by a strain of BCMV.

GGSV is seed and aphid transmitted (1) and shares some common features with guar symptomless virus (GSV) isolated from seeds in the United States (7). Investigators were unable to obtain symptoms in GSV-inoculated guar (7). Antiserum raised against GSV was found to react, in dot-blot indirect enzyme-linked immunosorbent assay (ELISA), with infected guar from southern Africa, but several differences between GGSV and GSV, with respect to transmissibility and host range, suggest that the two potyviruses, even though related, are different (1). Symptom studies on *P. vulgaris* previously undertaken, tentatively indicated that GGSV was related to an isolate of BCMV (1). Recent studies have shown that BCMV strains should be separated into two distinct potyvirus groups (12). BCMV serotype A strains NL3, NL5, NL8, and TN 1 have been named bean common mosaic necrosis virus (BCMNV), which may cause temperature insensitive necrosis in bean cultivars possessing the dominant *I* gene. The second group of isolates were maintained as BCMV serotype B along with a group of reclassified potyviruses. This investigation establishes GGSV as the causal agent of green-sterile disease, further characterizes the GGSV, and determines whether GGSV is a strain of BCMV or indeed a new guar legume potyvirus. Serological tests with BCMV and BCMNV are presented as well as comparisons with BCMV strains using differential bean host tests.

MATERIALS AND METHODS

Transmission and symptomatology. GGSV and a local strain of BCMV, BCMV-SA (5), were compared by symptoms produced in mechanically inoculated differential bean cultivars Diacol Calima, Monroe, Sanilac, Top Crop, Nep 2, Amanda, and Peru 0257, at temperatures of 25 C and 30 C. The presence of the dominant *I* gene or strain-specific recessive genes in the bean hosts is indicated in Table 1. All inoculations were performed with leaf tissue (either guar or inoculated beans) macerated in 0.01 M phosphate buffer, pH 7.0 with carborundum.

Further diagnostic hosts, *Nicotiana benthamiana* Domin., *N. tabacum* L., *Chenopodium quinoa* Willd., *Vigna radiata* (L.) (mungbean), *Cucumis sativus* L., *Lycopersicon esculentum* Mill. and *Glycine max* (L.) Merr. were mechanically inoculated with GGSV.

Differential bean cultivars, possessing the recessive or dominant alleles of the

Table 1. Comparison of GGSV[a] with a local strain of BCMV[b] using differential bean cultivars

P. vulgaris cultivars	Host resistance genes	Reaction of GGSV		Reaction of a BCMV-SA[c]
		25C	30C	
Monroe	bc-1^2, bc-2^2 (I^+I^+)[d]	+[e]	lc[f]	−[g]
Nep 2	II[h] gene	−	+	+/− n[i]
Sanilac	bc-2 (I^+I^+)	chl[j]	chl	++[k]
Peru 0257	II gene	−	−	+/− n
Amanda	II gene	−	lc	−
Diacol Calima	No R genes	+	+	++
Top Crop	II gene	+	+	−

[a] Guar green-sterile virus.
[b] Bean common mosaic virus.
[c] Local bean common mosaic virus (BCMV-SA) strain (pathogenicity group Va; BCMV serotype).
[d] Recessive alleles of the necrosis gene.
[e] Local vein necrosis on primary inoculated leaves.
[f] Leaf curl.
[g] No symptoms.
[h] Dominant alleles of the necrosis gene.
[i] Systemic necrosis — temperature dependent.
[j] Chlorosis on inoculated leaves.
[k] Systemic symptoms.

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necrosis gene (3,4,14) (Table 2), were mechanically inoculated with sap (from field guar affected by green-sterile disease). Symptoms were observed on primary, secondary, and tertiary trifoliate leaves. In order to ascertain whether a mixture of virus strains was present, primary and tertiary trifoliate leaves (which either showed symptoms or were positive when tested with the potyvirus monoclonal antibody PTY1 [10]) were back-inoculated onto a set of virus-free bean differentials.

GGSV-inoculated cv. Dubbele Witte was used in virus reisolation experiments. After the third passage a potyvirus extraction (6) was performed and negatively stained virions were observed with the electron microscope as previously described (1). Seeds from Dubbele Witte mechanically inoculated with GGSV were germinated; primary and tertiary leaves were tested with the monoclonal antibody PTY-1 and checked for the presence of potyvirus particles by transmission electron microscopy. Guar plants also were back-inoculated with sap from infected Dubbele Witte primary and tertiary leaves, and Dubbele Witte was back-inoculated with guar and macerated *N. benthamiana* leaves that had previously been inoculated with a purified GGSV preparation.

Serology. Bean indicator plants, cvs. Monroe, Dubbele Witte, and Black Turtle I, and guar cultivars inoculated with purified GGSV, were tested for the presence of potyvirus antigen in dot-blot indirect ELISA (8) with the potyvirus monoclonal antibody PTY-1 (10) as well as antisera raised against GGSV, BCMV-SA, BCMNV-NL3, BCMNV-NL5, and BCMV-NY15 strains. The NL3 antiserum detects all known BCMV and BCMNV isolates, NY15 antiserum detects BCMV isolates that do not produce systemic necrosis on bean cultivars containing the dominant *I* gene, and the NL5 antiserum only reacts against isolates of BCMNV. Two monoclonals MA bc-I-3 (specific for BCMNV-NL2, -NL3, -NL5, and -NL8) and MA B-1-1A4-C6 (reacts to all BCMV and BCMNV strains and a few other potyviruses) also were used in these serological tests.

Field-collected green-sterile guar plants, guar plants from germinated infected seed, Dubbele Witte grown from seed (collected from GGSV-inoculated Dubbele Witte), the set of bean differentials inoculated with sap from green-sterile guar (primary and tertiary trifoliate leaves), and back-inoculated bean differentials were all tested for virus antigen in indirect plate ELISA (15) using the monoclonal antibody PTY1. In all ELISA healthy bean and guar plants were used as negative controls and positive purified virus controls included GGSV, PVY, BCMV-SA, BCMNV-NL3, and BCMV-NY15.

Reproduction of disease in guar. Guar cvs. ZDPS, Tx-79-2741, and SL100 were mechanically inoculated with a sucrose gradient purified GGSV preparation (6). Comparisons were also made with similar guar cultivars inoculated with local potato virus Y strains (PVY-0, PVY-CB3, PVY-N) and BCMV-SA (5). Furthermore, germination of necrotic guar seeds from infected pods (1) was attempted in order to detect the appearance of virus symptoms.

Polyacrylamide gel electrophoresis (PAGE). GGSV was purified from guar (9) and purified viral antigen was run on denaturing PAGE (11). Electrophoresis was performed at 40 mA on 5% stacking and 12.5% resolving gels.

Western blotting. Protein bands from the gels were transferred onto Hybond-C nitrocellulose membranes (Amersham) and probed with BCMV-SA and GGSV antisera.

Nucleic acid preparation. Two hundred µl of a GGSV virion preparation was mixed with an equal volume of dissociation buffer (2% w/v sodium dodecyl sulphate, 2 mM EDTA, 20 mM Tris-HCl, pH 8) and incubated at 60 C for

Table 2. Reaction[a] of bean cultivars possessing the recessive or dominant alleles of the necrosis gene[b] (used to differentiate BCMNV[c] and BCMV[d] strains) to inoculation with GGSV[e]

Cultivars[f]	Pathogenic groups of BCMV[a]										Reaction[a] of GGSV
	I	II	III	IVa	IVb	Va	Vb	VIa	VIb	VII	
Dubbele Witte	+	+	+	+	+	+	+	+	+	+	(nm)[g]
Stringless Green Refugee	+	+	+	+	+	+	+	+	+	+	−
Redlands Greenleaf-C	−	+	−	+	+	+t	+	+t	+	+	−
Puregold Wax	−	+	−	+	+	+t	+	+t	+	+	−
Imuna	−	+t	−	+	+	+t	t	+t	+	+	−
Redlands Greenleaf-B	−	−	−	+	+	−	−	+	+	+	−
Great Northern 123	−	−	−	+	+	−	−	+t	+t	+	−
Sanilac	−	−	+	−	−	+	+	+	+	−	−
Red Mexican-34	−	−	+	−	−	+	+	+	+	−	−
Pinto 114	−	−	−	−	−	+	+	+	+	−	−
Monroe	−	−	−	−	−	−	−	−	−	+	−
Great Northern 31	−	−	−	−	−	−	−	−	−	+	−
Red Mexican-35	−	−	−	−	−	−	−	−	−	+	−
IVT 7214	−	−	−	−	−	−	−	−	−	−	−
Cultivars[h]											
Widusa	−	−	+n	−	+n	−	−	+n	+n	−	−
Black Turtle Soup I	−	−	+n	−	+n	−	−	+n	+n	−	+n
Jubila	−	−	−	−	+n	−	+n	+n	+n	−	−
Top Crop	−	−	−	−	+n	−	+n	+n	+n	−	−
Improved Tendergreen	−	−	−	−	−	−	+n	+n	+n	−	−
Amanda	−	−	−	−	−	−	−	−	+n	−	−

[a]Symptoms: + = susceptible, systemic mosaic; − = resistant, no systemic symptoms, virus not recovered from uninoculated leaves by back inoculation; +t = susceptible, tolerant, systemic symptoms questionable/weak, virus recovered from uninoculated leaves by back inoculation onto Dubbele Witte; +n = susceptible, sensitive, systemic necrosis not temperature dependent; −n = susceptible resistant, temperature dependent, variable systemic necrosis, i.e. some plants die, not all. T° = 20–22C min; 25–27 C max.
[b]According to Drijfhout (3,4).
[c]Bean common mosaic necrosis virus.
[d]Bean common mosaic virus.
[e]Guar green-sterile virus.
[f]Recessive alleles (I^+I^+) of the necrosis gene.
[g]No typical mosaic pattern but systemic chlorosis and leaf curl.
[h]Cultivars with dominant alleles (II) of the necrosis gene.

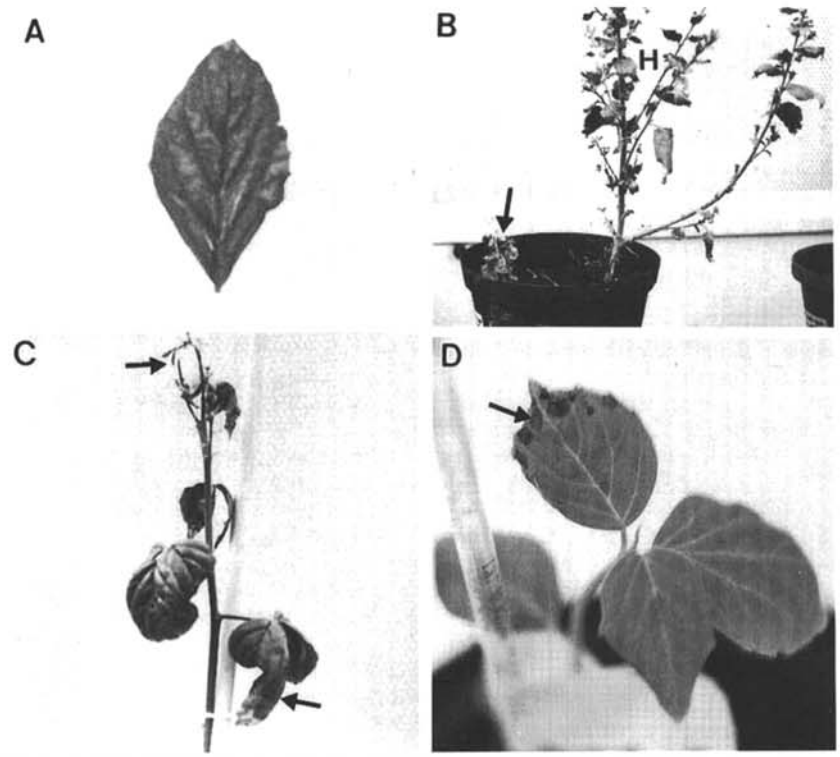

Fig. 1. Symptoms on guar infected with green-sterile disease and tobacco plants inoculated with guar green-sterile virus (GGSV). **(A)** Field-infected guar leaf exhibiting chlorotis symptoms. **(B)** *N. benthamiana* mechanically inoculated with GGSV. Note systemic necrosis (arrow). H = healthy plants. **(C)** Guar, inoculated with a purified extraction of GGSV, exhibiting leaf rolling, chlorosis, localized necrosis, and sterility (arrows). **(D)** Localized necrosis (arrow) on guar leaves from germinated infected seed.

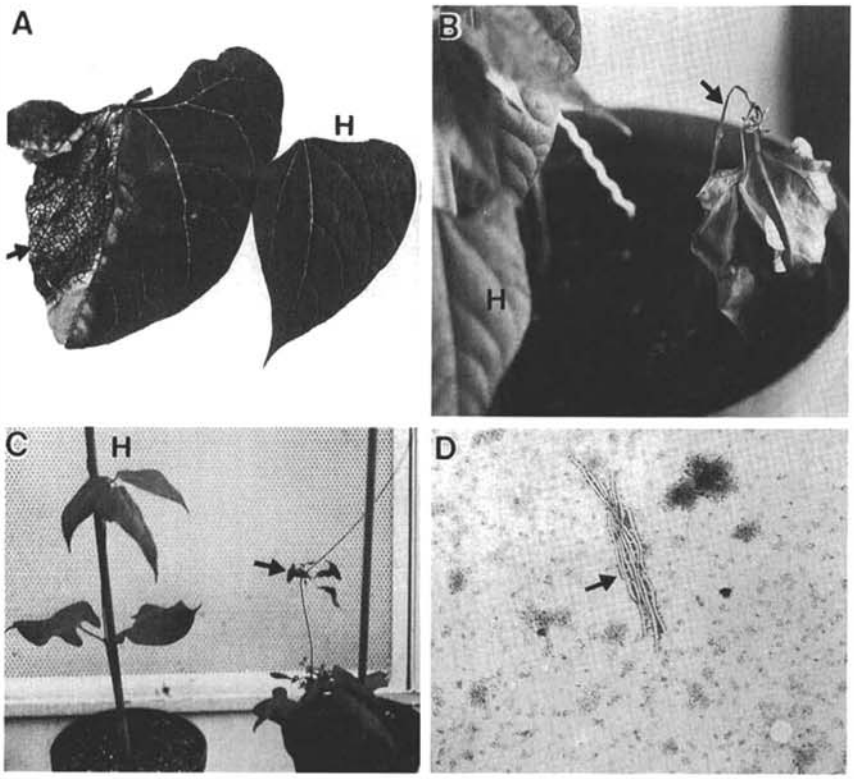

Fig. 2. Symptoms on inoculated *Phaseolus vulgaris* cultivars. **(A)** Black Turtle Soup I, exhibiting vein necrosis and chlorosis (arrow) on primary inoculated leaves. H = healthy leaf. **(B)**. Black Turtle Soup I, showing systemic necrosis and wilt (arrow). H = Healthy plant. **(C)** Dubbele Witte inoculated with GGSV-inoculated guar. Note leaf rolling and reduction in leaf size (arrow). H = Healthy plant. **(D)** Potyvirus particles observed in purifications from GGSV-inoculated Dubbele Witte and Black Turtle Soup I bean cultivars.

15 min. The aqueous phase was extracted with phenol/chloroform/isoamylalcohol (25:24:1) and the nucleic acid was precipitated using standard techniques of ethanol precipitation and centrifugation. The pellet was resuspended in TE buffer (0.01 M Tris, 0.001 M EDTA, pH 8.0) and run on a 1% agarose gel alongside RNA markers (Boehringer Mannheim, Germany), for size comparison. The nucleic acid preparation was subjected to RNase (10 μg/ml) treatment to confirm the RNA nature of the nucleic acid.

RESULTS

Transmission and symptomatology. No symptoms were observed on tomato, mungbean, *C. quinoa,* and *N. tabacum* mechanically inoculated with a virus preparation from field-infected guar. Soybean primary inoculated leaves exhibited localized necrosis and general chlorosis but no systemic spread was observed. Serological tests with antisera against GGSV, BCMV-SA, BCMNV-NL3, and BCMV-NY15 confirmed these negative results. *N. benthamiana* exhibited chlorotic symptoms after inoculation with GGSV and, after the third or fourth leaf stage, showed systemic necrosis, wilted, and died (Fig. 1B). Guar plants inoculated with a purified GGSV preparation exhibited leaf rolling, chlorosis, and localized necrosis on inoculated leaves (Fig. 1C). Seeds from infected guar pods were seldom able to germinate but upon germination the leaves occasionally exhibited localized necrosis (Fig. 1D) and chlorosis similar to that observed on field-collected and GGSV-inoculated guar.

Guar cvs. ZDPS, SL100, and TX-79-2741 (1) inoculated with PVY-0 or PVY-N exhibited foliar pinpoint chlorotic local lesions on inoculated leaves only. SL100 inoculated with PVY-CB3 exhibited chlorosis. Those guar plants inoculated with BCMV-SA showed a pale yellow mosaic and localized necrosis on inoculated leaves.

No systemic necrosis was noted on any of the bean cultivars inoculated with GGSV (Table 1). Comparisons with BCMV-SA strain (pathogenicity group Va) (3,4) also were undertaken. Both cvs. Diacol Calima and Top Crop exhibited local vein necrosis and chlorosis on GGSV-inoculated primary leaves only, whereas BCMV-SA showed systemic symptoms on Diacol Calima. Other differences between GGSV and BCMV-SA also were noted: BCMV-SA caused temperature dependent systemic necrosis in Peru 0257 whereas GGSV-inoculated cultivators exhibited no such symptoms (Table 1).

Symptoms, developed in bean differential cultivars inoculated with sap from guar affected by green-sterile disease, were compared with previously described reactions of BCMV pathogenicity groups (Table 2). Leaves inoculated with sap

from green-sterile guar or purified virions exhibited symptoms of localized necrosis followed by vein necrosis and chlorosis (Fig. 2A). Cultivars Great Northern 123 and 31, Sanilac, Red Mexican 34 and 35, Monroe, Top Crop, and Improved Tendergreen exhibited localized vein necrosis on primary inoculated leaves, but no systemic mosaic or other symptoms were observed on tertiary leaves of any cultivars except Dubbele Witte and Black Turtle Soup I. In the case of cvs. Puregold Wax, Stringless Green Refugee, and Redlands Greenleaf Band C, chlorotic veins were noted on primary inoculated leaves. Black Turtle Soup I exhibited systemic necrosis and the plants wilted and died (Fig. 2B). In the case of Dubbele Witte, the primary inoculated leaves exhibited vein necrosis and chlorosis, and the tertiary leaves exhibited chlorosis, localized vein necrosis, and sometimes leaf rolling. Dubbele Witte mechanically inoculated with sap from guar leaves (previously inoculated with GGSV) also exhibited systemic chlorosis, leaf rolling, and often a reduction in leaf size (Fig. 2C). Dubbele Witte plants back-inoculated with sap from macerated *N. benthamiana* leaves (previously inoculated with GGSV) exhibited systemic necrosis or chlorosis/localized necrosis. In no instances were mosaic symptoms observed. Potyvirus extractions from Dubbele Witte and Black Turtle Soup I confirmed the presence of virus particles in primary and tertiary leaves (Fig. 2D). Cultivars IVT 7214, Amanda, Widusa, Pinto 114, and Imuna exhibited no symptoms on primary or tertiary leaves. Back inoculations of sap from tertiary leaves of all bean cultivars (except Dubbele Witte and Black Turtle Soup I) onto the differential hosts did not result in symptoms, confirming lack of systemic spread in these host cultivars.

Primary and tertiary leaves from cultivars exhibiting symptoms on primary inoculated leaves were used in back inoculations onto Dubbele Witte in order to test for a mixture of strains. In all cases, symptoms of vein necrosis and chlorosis on primary leaves, and necrosis, chlorosis, and sometimes leaf rolling on tertiary leaves similar to that observed in primary inoculations, were observed in Dubbele Witte.

Serology. Field-infected green-sterile guar cvs. ZDPS, Lewis, and TX-79-2741 all tested positive with potyvirus monoclonal PTY-1 antibody (10), GGSV antiserum (1), and antisera against BCMV-SA, BCMNV-NL3, and BCMV-NY15. A positive reaction was also achieved with monoclonal MAB-1-1A4-C6 (reacts to all BCMV and BCMNV strains and a few other potyviruses) but not with MA bc-I-3, which is specific for BCMNV strains NL2, NL3, NL5, and NL8. Similar positive results were obtained with the above-mentioned antisera and guar cultivars inoculated with a pure GSSV preparation, as well as guar plants germinated from seeds from infected pods. Presence of potyvirus antigen was demonstrated in guar plants mechanically inoculated with BCMV-SA, PVY-0, and PVY-N (ELISAs were weakly positive with the potyvirus monoclonal PTY-1) but no cross-reactivity was obtained with the GGSV antiserum. Guar cv. SL100 inoculated with PVYC-B3 tested strongly positive for all potyvirus antisera. *N. benthamiana,* exhibiting systemic wilt after inoculation with sap from green-sterile diseased guar, reacted positively with the potyvirus monoclonal PTY-1, as did all bean cultivar primary leaves exhibitng symptoms. Tertiary leaves of Dubbele Witte and Black Turtle Soup I tested positive for potyvirus antigen, but all other bean-cultivar symptomless tertiary leaves did not show any reactivity with the monoclonal PTY1 antiserum. The differential bean hosts (used in back inoculations with sap from tertiary leaves) were symptomless and tested negative for potyvirus antiserum, again confirming lack of systemic spread, except in the case of Dubbele Witte and Black Turtle Soup I. Seeds from symptomless bean cultivars inoculated with sap from green-sterile diseased guar (GGSV) were germinated, and seedlings tested negative with monoclonal PTY 1. Dubbele Witte back-inoculated with sap from infected *N. benthamiana,* guar, and BTSI was positive in PTY 1 serological tests. Guar plants back-inoculated with

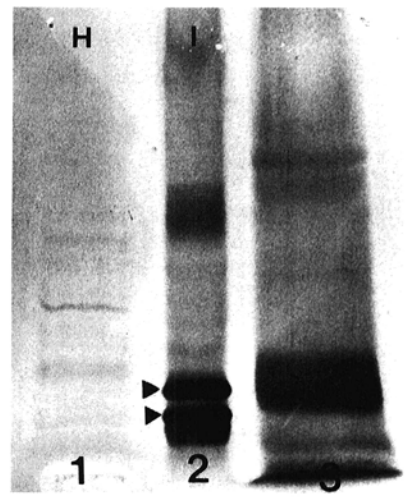

Fig. 4. Western blot of a virus extraction from infected guar, reacting to a local strain of bean common mosaic virus (BCMV-SA) antiserum. Immunobinding (arrows) is visible at the 32 and 34 KD bands. Lane 1 = healthy guar (H), Lane 2 = infected guar. Lane 3 = BCMV-SA.

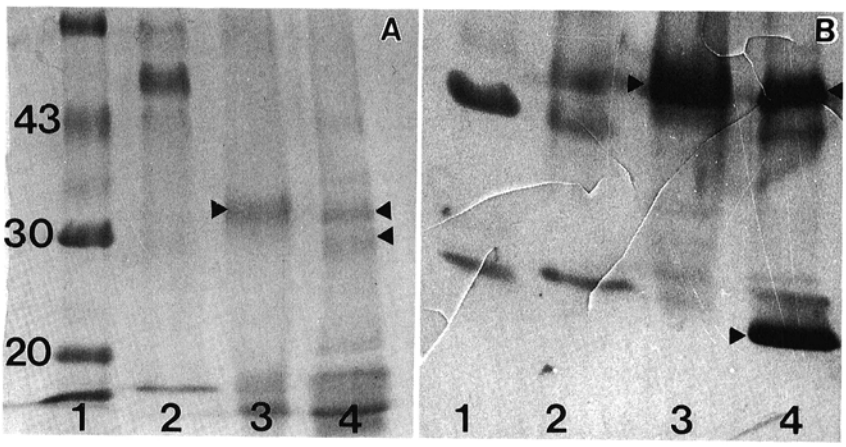

Fig. 3. (**A**) Denaturing 12.5% polyacrylamide gel electrophoresis of viral protein fractions isolated from green-sterile guar. Lane 1 = molecular weight markers. Lane 2 = healthy guar extraction. Lanes 3 & 4 = guar green-sterile virus (GGSV) protein bands of 32 and 34 KD (arrows). (**B**) Western blot, using GGSV antiserum, of purified virus extracts. Note strong binding of antiserum to the coat protein band at 34 KD and lower band of 12 KD apparent molecular weight (arrows). Lane 1 = molecular weight markers. Lane 2 = healthy guar. Lanes 3 & 4 = purified virus from green-sterile infected guar.

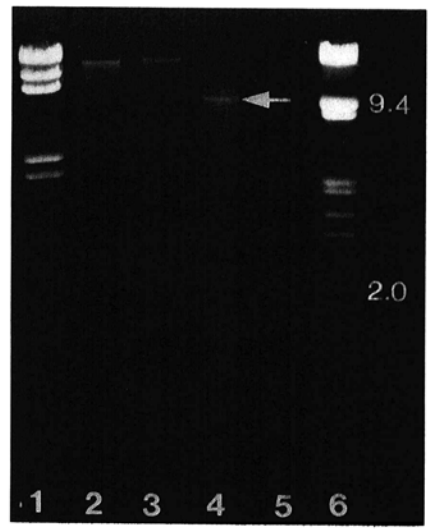

Fig. 5. 1% agarose gel of RNA isolated from guar green-sterile virus (GGSV). Lanes 1 & 6 = molecular weight markers. Lanes 2 & 3 = healthy guar plant nucleic acids. Lane 4 = RNA (9.4 Kb) isolated from GGSV, of apparent molecular weight 3.3×10^6 (arrow). Lane 5 = nucleic acid treated with RNAse.

Dubbele Witte also showed positive reactivity with the PTY 1 monoclonal.

PAGE and Western blotting. Three strong polypeptide bands with apparent molecular weights of 12, 32, and 34 KD (lanes 3 and 4) were detected in denaturing gels (Fig. 3A). Western blots probed with GGSV antiserum gave strong positive binding with the 34 KD protein from purified GGSV preparations (Fig. 3B) and less reactivity with the 32 KD polypeptide. Strong immunobinding to the 12 KD protein band also was obtained with the GGSV antiserum. Positive immunobinding to all three proteins was also observed when tested with BCMV-SA antiserum (Fig. 4)

Nucleic acid isolation. A single nucleic acid molecule of 3.3×10^6 apparent molecular weight (9.4 Kb) was isolated from purified virions (Fig. 5). The nucleic acid was shown to be RNA after digestion with RNAse (Fig. 5, lane 5).

DISCUSSION

GGSV was shown in previous studies to be associated with the guar green-sterile disease but unequivocal evidence that GGSV is the causal agent was not available (1). Past attempts to induce symptoms in guar by crude mechanical inoculation, or to produce plants with symptoms upon germination of seeds from infected pods, were unsuccessful. In this investigation, four guar plants, germinated from wrinkled seeds (from pods collected from field-infected guar), exhibited localized necrosis and yellow mottle similar to field-infected guar leaves. Furthermore, guar plants inoculated with a sucrose-gradient purified potyvirus preparation exhibited typical sterile and foliar symptoms. Both GGSV-inoculated plants and plants from infected seed showed a positive serological reaction with the potyvirus monoclonal PTY 1 confirming the presence of viral antigen. These reports, together with further potyvirus purifications from infected guar and mechanically inoculated bean cultivars, provide further evidence that the GGSV potyvirus is the causal agent of disease symptoms in guar. It would appear that GGSV causes leaf symptoms of localized necrosis and mild chlorosis similar to that induced with BCMV-SA and PVY strains N, O, and CB3. The more severe symptoms of sterility are manifested later at the onset of flowering.

Local vein necrosis and chlorosis on inoculated leaves is a common symptom in BCMV hosts with the dominant *I* gene (3,4,13). Similar symptoms on several differential bean cultivars, including Black Turtle Soup I, were achieved with GGSV inoculation and this previously led to the possible conclusion that GGSV may be a BCMV (1). Guar has been reported to be a host for BCMV (13). Serological tests, in this study, demonstrated that GGSV reacts with BCMV-SA antiserum, but in reciprocal tests GGSV antiserum reacted weakly with BCMV-SA (5). Furthermore, reactions on several bean cultivars differed between GGSV and BCMV-SA (Table 1). These results suggest that, although similarities in terms of symptomatology on bean differential hosts exist between GGSV and BCMV-SA, GGSV is probably not a strain of BCMV-SA. Positive serological reactivity between BCMNV-NL3 antisera and the monoclonal MA-B-1-1A4 and GGSV was achieved, but these antibodies will also detect a few other potyviruses (G. Mink, *personal communication*). BCMV-NY15 (which detects BCMV isolates) was also found to cross-react with GGSV. However, it is also noted that BCMV-NY15 isolates do not cause symptoms on Black Turtle Soup I, whereas GGSV did induce systemic necrosis in this bean cultivar. Comparisons of pathogenicity between GGSV and BCMV isolates, based on inoculation of bean cultivar differentials, demonstrated that GGSV does not fall into any of the existing BCMV pathogenicity groups. Except in Dubbele Witte and Black Turtle Soup I, no systemic mosaic symptoms, induced by many BCMV strains, were observed in this investigation. From these studies it can be concluded that GGSV serologically cross-reacts with several BCMV isolates, in particular the serotype B group, but this virus is not a strain of BCMV and is probably a new guar legume potyvirus. GGSV induces vein necrosis on inoculated leaves on several bean genotypes (both those with recessive and dominant alleles of the necrosis gene), but is unable to spread systemically in most bean cultivars. It is unlikely that a mixture of GGSV potyvirus strains exists, since similar symptoms of localized necrosis and chlorosis in Dubbele Witte and systemic necrosis in Black Turtle Soup I were always observed in back inoculations. Had there been a mixture of virus strains in the original field inoculum, some strains may have been selected during passaging and the symptoms altered. The only variation in symptoms was that in some cases GGSV-inoculated Dubbele Witte exhibited tertiary leaf rolling and occasionally the plants died, while in other instances they did not. However, this may be due to an environmental factor, or host-related factor such as inherent physiological differences.

PAGE and Western blotting confirmed the potyviral nature of GGSV, with a coat protein of 34KD. Two additional lower protein bands (12 and 32KD) are probably due to proteolytic degradation often observed in potyvirus preparations. The RNA species isolated from GGSV was found to be 9.4Kb (3.3×10^6 apparent molecular weight), typical of most potyviruses (2).

The guar green-sterile virus has typical properties of a potyvirus but would appear to be a new guar legume potyvirus. Its relationship with the guar symptomless virus (7) and BCMNV isolates has yet to be ascertained. Establishment of cDNA clones has recently been achieved and further characterization of this guar virus is underway.

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