A Strain of Peanut Mottle Virus Seedborne in Bambarra Groundnut

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ABSTRACT

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Peanut mottle virus (PMoV) was detected in nine of 95 bambarra groundnut (*Voandzeia subterranea*) plants grown in Florida from seed produced in Africa. This virus (PMoV-VS) induced systemic mosaic symptoms in cultivars of peanut (*Arachis hypogaea*), lima bean (*Phaseolus limensis*), and pea (*Pisum sativum*). PMoV-VS induced symptoms in differential bean (*Phaseolus vulgaris*) cultivars similar to those noted for an isolate of PMoV from the United States. PMoV-VS and PMoV induced very similar scroll, pinwheel, and laminated aggregate inclusions in infected leaf tissues. In immunodiffusion tests with PMoV-VS antiserum, precipitin lines of PMoV-VS and PMoV fused without spur formation. In reciprocal tests, homologous lines of PMoV spurred over those of PMoV-VS. Cross-reactions between PMoV-VS and PMoV were noted in reciprocal direct double-antibody-sandwich enzyme-linked immunosorbent assays and western blot tests.

Additional keywords: bean common mosaic virus, potyvirus group monoclonal antibody

Bambarra groundnut (*Voandzeia subterranea* (L.) Thouars), a drought-tolerant edible legume indigenous to tropical Africa, is now also cultivated as a subsistence crop in parts of Asia, Australia, and Latin America (8). Although relatively free from disease and insect problems, bambarra groundnut is susceptible to several viruses, including cowpea mottle (17), cowpea mild mottle (18), Voandzeia necrotic mosaic (10), and white clover mosaic (16) viruses and at least two potyviruses (2–4). The potyvirus described by Bock et al (4) from Tanzania, East Africa, is related serologically to peanut mottle virus (PMoV), while the one reported by Bird and Corbett (3) from Togo, West Africa, apparently is not (2). Neither potyvirus infected peanut (*Arachis hypogaea* L.).

We describe a seedborne potyvirus (PMoV-VS) from bambarra groundnut that infects peanut and is closely related serologically to other isolates of PMoV. Our virus (12) and the one from Togo (2) were detected in plants derived from seeds collected in Africa and sown in Florida and Maryland, respectively.

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MATERIALS AND METHODS

Sources of materials. Five accessions of bambarra groundnut seed imported in 1988 from the Genetics Resources Unit of the International Institute of Tropical Agriculture in Ibadan, Nigeria, under a U.S. Department of Agriculture permit were used throughout this investigation. R. Provvidenti (New York State Agricultural Experiment Station, Cornell University, Geneva) provided seed of bean (*Phaseolus vulgaris* L.) cultivars Black Turtle 1 and Black Turtle 2. F. J. Morales (Centro Internacional de Agricultura Tropical, Cali, Colombia) provided seed of Improved Tendergreen, Jubila, Topcrop, and Widusa bean. H. A. Scott (Department of Plant Pathology, University of Arkansas, Fayetteville) provided Black Valentine, Cherokee Wax, and Pinto bean seed, and C. W. Kuhn (Department of Plant Pathology, University of Georgia, Athens) provided seed of Bountiful, Kentucky Wonder, and Topcrop bean. The peanut seeds used throughout this investigation were indexed and found to be free of PMoV and peanut stripe virus (24).

Antisera and reference antigens of blackeye cowpea mosaic, papaya ringspot-type W, peanut mottle (21), peanut stripe, soybean mosaic, watermelon mosaic 2, and zucchini yellow mosaic viruses were provided by D. E. Purcifull and/or E. Hiebert (Department of Plant Pathology, University of Florida, Gainesville). J. W. Demski (Georgia Agricultural Experiment Station, University of Georgia, Griffin) provided the isolate of PMoV (PMoV-M) used in host range trials and certain serological tests. T. P. Pirone (Plant Pathology Department, University of Kentucky, Lexington) provided tobacco vein mottle viruses (antisera and reference antigens).

Inoculations. Test plants were dusted with 0.22-μm mesh Carborundum and then inoculated manually. Inocula were prepared by triturating leaf tissue in 0.05 M potassium phosphate buffer, pH 7.2. Systemic infections were confirmed serologically or by back inoculations in all host range trials. Pea (*Pisum sativum* L.) cultivars Dwarf Grey Sugar and Kerona and Kentucky Wonder Wax bean were routinely used in back inoculations. Serological confirmations were

made with PMoV antiserum in western blot or immunodiffusion tests.

Electron microscopy. Plant material was fixed for thin sectioning in 4% glutaraldehyde (in 0.1 M potassium phosphate buffer, pH 7.2), postfixed with 1% osmium tetroxide, dehydrated in an acetone series, and embedded in Spurr's epoxy resin. Thin sections were made with a diamond knife and stained with uranyl acetate and lead citrate.

Purification. Two weeks after inoculation, one of the virus isolates from an infected bambarra groundnut seedling (PMoV-VS) was purified from pea leaves as described by Xiong et al (21), except that Triton X-100 was omitted. The final virus preparation was resuspended in a 0.02 M Tris-HCl buffer, pH 8.0, and analyzed with a Beckman Model 25 spectrophotometer.

The molecular weights of PMoV-VS and PMoV-M protein subunits were estimated by electrophoresis of sodium dodecyl sulfate (SDS)-treated purified virus in 10% polyacrylamide gels (SDS-PAGE), as described by Hiebert and McDonald (11). The following markers were used for comparison: myosin (mol wt 200,000), phosphorylase A (94,000), bovine serum albumin (66,000), glutamate dehydrogenase (53,000), carbonic anhydrase (29,000), and capsid subunits of tobacco mosaic virus (17,500).

Serology. A rabbit was injected intramuscularly each week for 3 wk with 1.76-mg aliquots from the same preparation of purified PMoV-VS. For the first injection, the virus was emulsified in Freund's complete adjuvant (1:1, v/v). Freund's incomplete adjuvant was used in the two subsequent injections. Serum was collected weekly beginning 1 wk after the final injection.

SDS immunodiffusion tests were done as described by Purcifull and Batchelor (15). The diffusion medium consisted of 0.8% Noble agar, 0.5% SDS, and 1% sodium azide. Direct double-antibody-sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was performed as described by Zettler and Elliott (23). Immunoglobulins of PMoV-VS and PMoV were coated in wells at standard concentrations of 0.1 and 1.0 μg/ml, respectively. The A_{405nm} values obtained represent at least three replicated wells per sample per trial. All samples were extracts of pea leaf tissue diluted 1:10 or 1:100.

PMoV-VS and PMoV-M antigens, diluted 1:10, were tested in plate-trapped indirect ELISA (I-ELISA) tests (20,22) against 1:500 dilutions of the "potyvirus group" monoclonal antibody (Agdia, Inc., Elkhart, IN).

A rapid method was used to detect viral proteins on western blots. Minced tissue (1:5, w/v) was added to SDS extraction buffer (62.5 mM Tris buffer, pH 6.8, containing 2% SDS, 10% glycerol, and 5% mercaptoethanol) and placed in a boiling water bath for 90 sec. Extracts were run on SDS-PAGE minigels for 1–1.5 hr at 100-V constant voltage. Separated proteins were transferred to nitrocellulose membranes by electroblotting for 30 min (19). The blots were washed for 1 min in TTBS buffer (20 mM Tris buffer, pH 7.5, with 500 mM NaCl and 0.05% Tween-20), soaked for 15 min at 37 C in a 1:200 dilution of PMoV-VS or PMoV antiserum, washed twice for 3 min each time with TTBS buffer, and soaked for 15 min at 37 C in alkaline phosphatase-conjugated goat antirabbit immunoglobulin. The conjugate was detected with 0.3 mg/ml nitro blue tetrazolium and 0.15 mg/ml 5-bromo-4-chloro-3-indolyl phosphate in 0.1 M $NaHCO_3$ (pH 9.8) buffer containing 1.0 mM $MgCl_2$. The same markers used in SDS-PAGE trials were also used in western blots.

RESULTS

PMoV-VS was detected in two of eight, none of five, two of eight, four of 66, and one of eight greenhouse-grown bambarra groundnut seedlings derived from the five seed accessions TVSU-009, -119, -334, -702, and -992, respectively. Infected plants had foliar mosaic and distortion symptoms (Fig. 1) and were stunted relative to their noninfected counterparts.

The virus was manually transmitted to *Nicotiana benthamiana* Domin and to seven of the eight species of Leguminosae included in host range studies. PMoV-VS did not infect broad bean (*Vicia faba* L.) or any of the following species in other families: *Capsicum annuum* L., *Cucumis sativus* L., *Cucurbita pepo* L., *Cucurbita pepo* var. *melopepo* (L.) Alef., *Datura stramonium* L., *Gomphrena globosa* L., *Gossypium hirsutum* L., *Lycopersicon esculentum* Mill., *N. edwardsonii* Christie & D. W. Hall, *N. glutinosa* L., *N. rustica* L., *N. tabacum* L., *Passiflora edulis* Sims, and *Zinnia elegans* Jacq. PMoV-VS induced systemic mosaic symptoms in bambarra groundnut (TVSU-334), lima bean (*Phaseolus limensis* Macf. 'Jackson Wonder'), *Macroptilium lathyroides* (L.) Urban, peanut (cultivars Southern Runner and Sunrunner), and eight pea cultivars tested (Alaska, Dwarf Grey Sugar, Kerona, Little Marvel, Rondo, Sugar Daddy, Thomas Laxton, and Wando). Four cowpea (*Vigna unguiculata* (L.) Walp.) cultivars (Cream Elite, Knuckle Purple Hull, Lady Finger, and White Acre) were susceptible to PMoV-VS, whereas four others (Big Boy, Pink-eye Purple Hull, Ramshorn Blackeye, and Zipper Cream) were not.

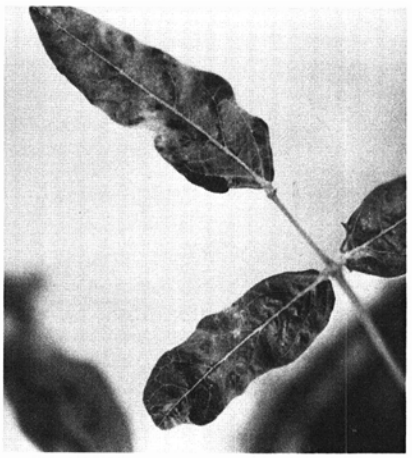

Fig. 1. Bambarra groundnut (*Voandzeia subterranea*) leaves showing mosaic and distortion symptoms induced by a seedborne potyvirus (PMoV-VS).

PMoV-VS and PMoV-M induced similar symptoms in 14 of 18 bean cultivars (Table 1). In Topcrop bean, both viruses induced local lesions like those described by Paguio and Kuhn (13) for the M2 strain of PMoV. Six days after inoculation, 100 lesions induced by PMoV-VS and PMoV-M averaged 1.38 and 0.94 mm^2, respectively, in area (ranges 0.4–2.5 and 0.3–2.0 mm^2, respectively).

Cylindrical inclusions were observed in thin sections of PMoV-VS and leaf tissues infected with the PMoV isolate studied by Xiong et al (21). Both viruses induced very similar subdivision-III cylindrical inclusions (9) consisting of scrolls, pinwheels, and laminated aggregates (Fig. 2).

An estimated yield of 0.18 mg of PMoV-VS per gram of pea tissue was obtained, and the $A_{260/280nm}$ ratio of the purified preparation was 1.29. A single electrophoretic component with an estimated molecular weight of 31,000–32,000 was found after SDS-PAGE analysis of purified virus. Similar

Table 1. Symptoms of bean (*Phaseolus vulgaris*) cultivars inoculated with isolates of peanut mottle virus from bambarra groundnut (PMoV-VS) and peanut (PMoV-M)

	Symptoms[a]	
Cultivar	**PMoV-VS**	**PMoV-M**
Black Turtle 1	NL, SN	NL, SN
Black Turtle 2	NL, SN	NL, SN
Black Valentine	CL	CL
Blue Lake	I	CL
Bountiful	SM	CL
Cherokee Wax	CL, SM	NL
Commodore	I	I
Dade Pole	NL, SN	NL, SN
Dwarf Horticulture	I	I
Harvester	I	I
Improved Tendergreen	NL	NL
Jubila	NL	NL
Kentucky Wonder	NL, SN	NL, SN
Pinto	NL	CL
Roma	I	I
Tendergreen	NL	NL
Topcrop	NL	NL
Widusa	NL, SN	NL, SN

[a]NL = necrotic local lesions, CL = chlorotic local lesions, SM = systemic mosaic or chlorosis, SN = systemic necrosis, I = insusceptible.

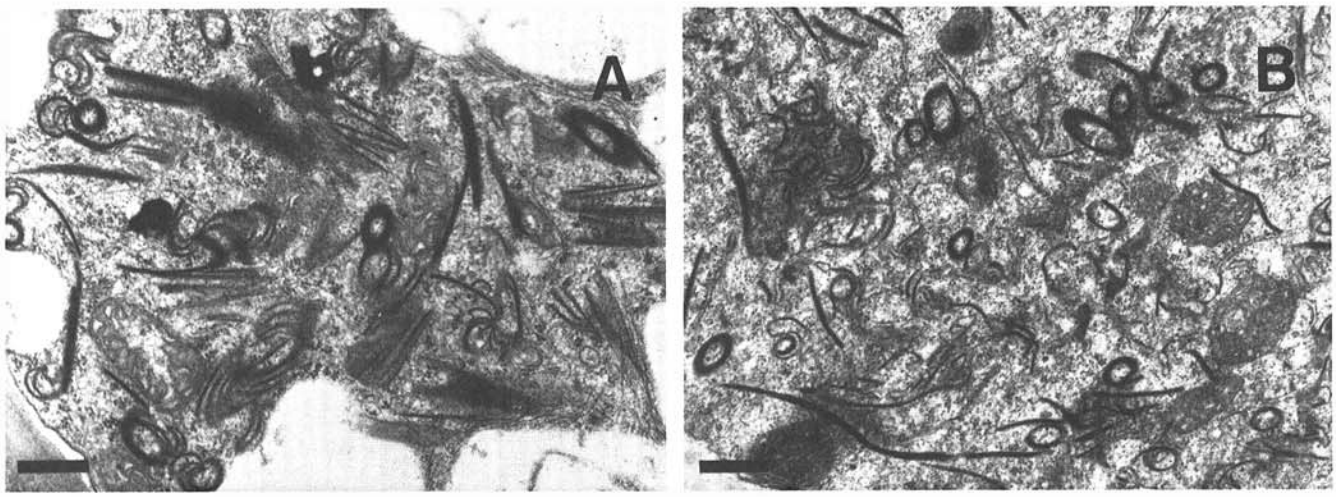

Fig. 2. Ultrastructure of leaf tissue of *Voandzeia subterranea* **(A)** and *Pisum sativum* **(B)** infected with a seedborne potyvirus (PMoV-VS) and with peanut mottle virus, respectively, showing laminated aggregates, pinwheels, and scrolls. Scale bar = 500 nm.

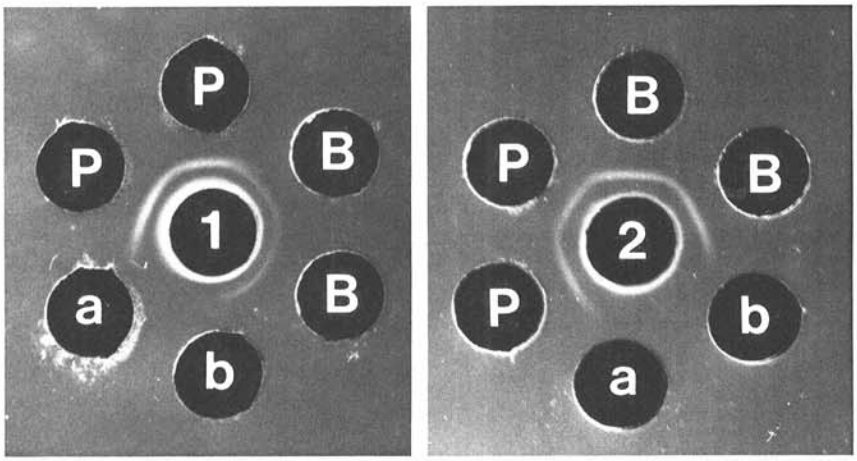

Fig. 3. Serological comparison of a seedborne potyvirus of *Voandzeia subterranea* (PMoV-VS) and peanut mottle virus (PMoV-M). Center wells 1 and 2 contained PMoV-M and PMoV-VS antiserum, respectively. Peripheral wells contained leaf extracts of peanut (*Arachis hypogaea*) infected with PMoV-M (P), leaf extracts of *V. subterranea* infected with PMoV-VS (B), and leaf extracts of healthy peanut (a) and healthy *V. subterranea* (b).

solitary bands with molecular weight 31,000–32,000 were noted for PMoV-M, potato Y virus, and tobacco vein mottle virus used for comparison.

Antiserum to PMoV-VS reacted with both its homologous antigen and with heterologous antigens of PMoV in immunodiffusion, ELISA, and western blot tests. Homologous precipitin lines of PMoV-VS fused without spur formations with the heterologous PMoV lines. In contrast, precipitin lines of PMoV spurred over those of PMoV-VS in reciprocal tests (Fig. 3). Homologous precipitin lines of zucchini yellow mosaic virus also spurred over relatively faint heterologous lines of PMoV-VS. In reciprocal tests, however, PMoV-VS did not react with zucchini yellow mosaic virus. No reactions were observed between PMoV-VS antigens and antisera to the following potyviruses: bean common mosaic (NL and common serotypes), bean yellow mosaic, bidens mottle, lettuce mosaic, papaya ringspot-type W, peanut stripe, soybean mosaic, and tobacco etch.

In reciprocal DAS-ELISA tests, positive A_{405nm} values for heterologous and homologous antigens were noted, regardless of whether PMoV-VS or PMoV antiserum was used. When PMoV-VS antiserum was used, values for PMoV-VS and PMoV-M antigens ranged from 0.448 to more than 2 and from 0.189 to more than 2, respectively (means 1.222 and 0.896, respectively). When PMoV antiserum was used, values for PMoV-VS and PMoV-M ranged from 0.254 to more than 2 and from 0.387 to 1.647, respectively (means 0.707 and 0.835, respectively). A_{405nm} values for extracts of healthy plants ranged from 0 to 0.289 (mean 0.156). Antisera of both viruses reacted with PMoV-VS and PMoV-M in western blots. The intensities of the bands were similar regardless of which antiserum or virus was tested.

The Agdia potyvirus group monoclonal antibody reacted in I-ELISA tests with the potyviruses dasheen mosaic, papaya ringspot-type W, watermelon mosaic 2, and zucchini yellow mosaic but not with PMoV-VS or PMoV-M. A_{405nm} values ranged from 0.000 to 0.037 (means 0.014 and 0.006 for PMoV-VS and PMoV-M, respectively), whereas values ranging from 0.440 to 3.500 were noted for the other four potyviruses tested in the same experiments (respective means 0.713, 1.105, 2.440, and 2.063). Mean A_{405nm} values for homologous polyclonal antisera of PMoV-VS and PMoV-M were 1.316 and 1.398, respectively. A_{405nm} values for healthy plant extracts used as controls ranged from 0.000 to 0.024.

DISCUSSION

PMoV-VS appears to be a strain of PMoV, based on serological results and similarities in cylindrical inclusion morphologies, host range, and symptoms induced in differential bean cultivars. Thirteen differential bean cultivars tested for susceptibility to PMoV by Provvidenti and Chirko (14) were also tested for susceptibility to PMoV-VS and PMoV-M in this study. Except for Cherokee Wax, which developed systemic symptoms with PMoV-VS, the reactions reported by Provvidenti and Chirko (14) were noted for both viruses.

The slight unilateral differences between PMoV-VS and PMoV antigens noted in immunodiffusion tests are unlikely to constitute sufficient grounds for considering the two strains distinct potyviruses. PMoV-VS and PMoV-M also resemble each other in that neither appears to have the epitope common to other potyviruses which reacts against the Agdia potyvirus group antibody. Although Bock et al (4) were unable to infect peanut with their bambarra groundnut virus isolate, they considered it to be a strain of PMoV based on the close serological relationships noted in liquid precipitin tests.

Of the five strains of PMoV described by Paguio and Kuhn (13), the M2 strain most closely resembles PMoV-VS, based on the mild mottle symptoms they induce in peanut and the size of lesions (about

1 mm^2) induced on Topcrop bean. M2 is the most prevalent strain of PMoV in Georgia (13) and elsewhere. Only the M2 strain has been detected in Africa and Australia (4,5).

This is the first report of seed transmission of PMoV in bambarra groundnut. The only other potyvirus previously reported to be seedborne in this host is the one studied in Maryland (2,3). Unlike that virus, however, PMoV-VS infects pea and peanut and is closely related serologically to PMoV. Cowpea mottle virus, a spherical virus, is also seed-transmitted in bambarra groundnut (17) but was not detected in this investigation.

PMoV-VS was seed-transmitted in bambarra groundnut at relatively high rates (about 10%), which would enable it to be perpetuated indefinitely in planting stock, as with PMoV in peanut. Thus, bambarra groundnut could also be a potential reservoir of PMoV inoculum for other legume crops, such as soybeans (7), grown in association with it. PMoV-VS was detected in four of the five Nigerian germ plasm accessions, which represented material grown in two locations in Nigeria and one in Burkina Faso. Likewise, isolates of the virus studied by Bock et al (4) were detected in bambarra groundnut plantings 700 km from one another and at different elevations (500 and 1,200 m). PMoV is also seed-transmitted in bean (1) and cowpea (6) but at much lower rates than in either bambarra groundnut or peanut. *M. lathyroides,* a legume not previously reported as a suscept of PMoV, was infected by PMoV-VS. Thus, this common perennial weed in tropical and subtropical countries could serve as a potential inoculum reservoir of PMoV and its variants.

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