Cowpea Viruses in Senegal, West Africa: Identification, Distribution, Seed Transmission, and Sources of Genetic Resistance

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ABSTRACT

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Viral diseases of cowpea (*Vigna unguiculata* subsp. *unguiculata*) in Senegal were surveyed during the rainy seasons of 1990 and 1991. Sixty-six symptomatic plant samples from five production areas were assayed for seven viruses by enzyme-linked immunosorbent assay (ELISA). Four recognized viruses, cowpea aphid-borne mosaic potyvirus (CABMV), cowpea mottle carmovirus (CPMoV), cowpea severe mosaic comovirus (CSMV), and southern bean mosaic sobemovirus (SBMV), were detected in 34, 2, 1, and 1 samples, respectively. All are seed-transmissible in cowpea. Variants of an unknown potyvirus were also detected in 21 samples. These variants occurred principally in new, improved CABMV-resistant cowpea genotypes, and their combined incidence in plant samples was exceeded only by CABMV. Isolates of the unknown potyvirus were seedborne in Senegal cowpea lines and were nonpersistently transmitted by the cowpea aphid, *Aphis craccivora*. Selected seedborne isolates of this potyvirus were distinguishable principally by differentially resistant cowpea genotypes and by either weak (isolate V1-1) or strong (isolate V17-14) reactions to potyvirus-selective monoclonal antibodies. Of 35 cowpea genotypes tested as possible sources of resistance to the unknown potyvirus, six (TVU-401, TVU-408P2, TVU-1000, TVU-1016-1, TVU-1582, and White Acre-BVR) were resistant to all isolates of the virus. These genotypes have been included in the Senegal cowpea breeding program for development of virus-resistant cultivars.

Cowpea (*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*) is second in importance only to groundnut among Senegal legume crops. Cowpea is grown on some 63,000 ha annually in Senegal, with an annual production of 18,000,000 kg. Since the average commercial yield of 280 kg/ha represents only 14–28% of the 1,000–2,000 kg/ha yields in experimental fields (19), doubling the cowpea yield in Senegal should be readily achievable. Several factors contribute to the low yields, but viral diseases are considered a major limitation.

Among cowpea viruses reported in West Africa (14,15,17,19,20,22), cowpea mosaic comovirus (CPMV) (= cowpea yellow mosaic) (20) and cowpea aphid-borne mosaic potyvirus (CABMV) are considered economically most important. Other viruses known to occur in West Africa include cowpea mottle carmovirus (CPMoV) (1,18) and southern bean mosaic sobemovirus (SBMV) (7,13). Cowpea mild mottle carlavirus (CMMV), cowpea severe mosaic comovirus (CSMV), and cucumber mosaic cucumovirus (CMV) had been previously detected in seed lots from Burkina Faso, Nigeria, Senegal, and Ghana, respectively (8).

Seedborne viruses were considered a major constraint to cowpea yield in Senegal farm fields (D. G. Gaikwad, Annual Report, Cowpea Pathology, ISRA/CNRA, Bambey, Senegal, *unpublished*). Cowpea breeding lines IS86-275N (released in 1992 as cv. Mouride) and IS86-283-15N were recently developed to increase sustainable cowpea production in Senegal. These lines were resistant to CABMV, bacterial blight (*Xanthomonas campestris* pv. *vignicola* (Burkholder) Dye), storage weevil (*Callosobruchus maculatus* (Fabricius)), striga (*Striga gesnerioides* (Willd.) Vatke), and drought (19). However, viral diseases in field trials of these lines in 1989–90 suggested the incidence of unrecognized indigenous viruses or perhaps undescribed pathotypes of CABMV. The present study was conducted to isolate and partially characterize these viruses and to identify resistant cowpea genotypes for use in developing virus-resistant cultivars (16).

MATERIALS AND METHODS

Field survey and collection of viral isolates. During the rainy seasons of 1990 and 1991, fields in the five cowpea production areas of Senegal were surveyed for viral diseases. Leaf samples were collected from symptomatic plants in farm fields and experimental plantings. Samples were desiccated over $CaCl_2$ and deposited in the Virology Laboratory, USDA ARS, Department of Botany and Plant Pathology, Oregon State University, Corvallis, for investigation. Senegal field isolates V-1 and V-2 originated in naturally infected cowpea plants in Kolda, and isolates V-17 and V-54 originated in naturally infected cowpea plants in Diourbel.

Disease reactions, seed transmission, and host-range tests. Seedling plants of five advanced cowpea lines/cultivars were dusted with silicon carbide powder and mechanically inoculated with four Senegal viral field isolates, to reproduce the previously observed disease symptoms. Plants were mechanically inoculated twice at 7-day intervals and maintained under both field and screenhouse conditions near Bambey. Insecticide was applied as needed to control potential insect vectors. Disease incidence in field plots (% symptomatic plants) was recorded biweekly from 7 to 45 days after inoculation.

Seed was harvested from plants in the above test and sent to Corvallis, Oregon, where it was tested for seedborne virus by growing out seedlings in insect-free glasshouses. Seedling infection was estimated by visual inspection and results were verified by enzyme-linked immunosorbent assay (ELISA). Two-week-old symptomatic seedlings were assayed for seedborne potyviruses by direct antigen coating (DAC) ELISA with Agdia PTY and II-197 monoclonal antibodies (MAbs).

Potyviral isolates derived from individual infected seedlings of cultivars 58-57 and Mougne (from inoculated, screenhouse-grown mother plants) were assigned distinct subnumbers (for example V17-2) and referred to as "PTY+" (detectable by PTY MAb). Reference cultures were preserved in desiccated infected tissues at −30 C and in infected seed of selected cowpea cultivars. Selected plant species and strategic cowpea genotypes were tested for sus-

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Table 1. Viruses detected by DAC-ELISA in field samples of cowpea collected in five districts of Senegal (West Africa)

District surveyed/ sampled	No. samples collected	No. samples reacting positively with antiviral antisera or potyvirus MAb							
		BlCMV[a]	CABMV	CMV	CPMoV	CPMV	CSMV	SBMV	MAb II-197
Diourbel	28	—[b]	10	—	1	—	1	—	11[c]
Kolda	4	—	1	—	—	—	—	—	2
Louga	20	—	10	—	1	—	—	1	7
Tambacounda	10	—	9	—	—	—	—	—	1
Thies	4	—	4	—	—	—	—	—	—
Total	66	—	34	—	2	—	1	1	21

[a]Samples were tested against antisera to blackeye cowpea mosaic (BlCMV) and cowpea aphid-borne mosaic (CABMV) potyviruses, cucumber mosaic cucumovirus (CMV), cowpea mottle carmovirus (CPMoV), cowpea mosaic (CPMV) and cowpea severe mosaic (CSMV) comoviruses, and southern bean mosaic sobemovirus (SBMV), and against potyvirus monoclonal antibody PTY II-197.
[b]— Indicates virus not detected by ELISA.
[c]Samples reacting to monoclonal antibody PTY II-197 contained no virus detectable by other antiviral polyclonal antisera.

Table 2. Disease incidence (mechanical inoculations) and seed transmission associated with Senegal potyvirus PTY+ field isolates

Virus isolate	Cultivar/ line	Disease incidence[a] (%)	No. seeds germinated/ no. planted	Seed transmission[b]	
				Incidence	(%)
V-1	Baye Ngagne	100	37/50	1/37	3
	IS86-275N	61	41/50	0/41	0
	IS86-283-15	8	29/50	0/29	0
	Mougne	97	46/50	2/46	4
	58-57	100	46/50	6/46	13
V-2	Baye Ngagne	100	46/100	0/46	0
	IS86-275N	47	37/100	0/37	0
	IS86-283-15	18	44/100	0/44	0
	Mougne	100	92/100	1/92	1
	58-57	55	83/100	7/83	8
V-17	Baye Ngagne	81	35/50	0/35	0
	IS86-275N	50	40/50	2/40	5
	IS86-283-15	52	23/50	0/23	0
	Mougne	97	47/50	0/47	0
	58-57	93	43/50	13/43	30
V-54	Baye Ngagne	86	27/50	0/27	0
	IS86-275N	88	40/50	2/40	5
	IS86-283-15	82	28/50	0/28	0
	Mougne	97	49/50	0/49	0
	58-57	89	44/50	1/44	2

[a]Experiments conducted at the Institute of Agricultural Research, Bambey, Senegal.
[b]Seeds taken from potyvirus-inoculated plants, Bambey screenhouse plots. Experiments conducted in greenhouses, Corvallis, Oregon. PTY+ = plants reacted positively to Agdia potyvirus monoclonal antibody.

ceptibility to five selected PTY+ isolates. Eight to 10 plants of each species/genotype were mechanically inoculated under glasshouse conditions (temperature 28–30 C, 14-hr photoperiod, and solar irradiant equivalence of 87–121 kJm^{-2} day^{-1}). Five weeks after inoculation, symptomless plants were tested for asymptomatic infections by DAC-ELISA and Agdia PTY MAb.

Aphid transmission. Aphid-transmissibility of PTY+ isolates V1-1 and V17-14 was tested using an *Aphis craccivora* Koch colony reared on healthy cowpea plants. Plant-to-plant transmission was carried out as follows: Groups of mixed fourth and fifth instar apterae were removed from rearing plants, starved for 2 hr, then deposited on detached virus-infected cowpea leaves and allowed 3–4 min periods for acquisition access. Apterae found in a feeding position were transferred with a fine camel-hair brush to healthy test plants of Senegal cowpea cultivar 58-57. A total of 26–35 test plants were inoculated with each virus isolate using three aphids per test plant. The aphids were allowed to feed overnight on test plants before treatment with synthetic pyrethroid insecticide. Inoculated plants were observed for symptom development for 4 wk after aphid inoculations. Symptomless plants were assayed by DAC-ELISA using PTY MAb. All transmission tests were repeated.

Serology. Field-collected samples from Senegal were tested by either double antibody sandwich (DAS) ELISA (5,6) or by DAC-ELISA (8) for the possible presence of seven seedborne viruses, including blackeye cowpea mosaic potyvirus (BlCMV), CABMV, CPMV, CSMV, CPMoV, CMV, and SBMV. The samples were also tested by DAC-ELISA against either potyvirus-selective Agdia MAb PTY (11) or anti-BCMV MAb II-197 (21).

Antisera to BlCMV and SBMV were kindly provided by Cedric Kuhn (University of Georgia, Athens), and antisera to CPMV and CSMV were kindly provided by O. W. Barnett (Clemson University, Clemson, SC). The MAb II-197 was kindly provided by G. I. Mink (Washington State University, Prosser), and MAb PTY (11) was obtained from Agdia Inc., Elkhart, IN. The other antisera were produced by the Virology Laboratory, USDA ARS, Department of Botany and Plant Pathology, Oregon State University.

Virus isolates were derived through seed transmission and were tested as infected-plant extracts by DAS-ELISA against immunoglobulin G (IgG) to BlCMV, CABMV, pea seedborne mosaic potyvirus (PSbMV), and CMV (as possible contaminants), and by DAC-ELISA against antisera to several potyviruses, including BlCMV, CABMV, clover yellow vein virus (CYVV), peanut mottle virus (PeMoV), peanut stripe virus (PStV), PSbMV, white lupin mosaic virus (WLMV), and also against PTY MAbs. Isolates reactive in DAC-ELISA to only the monoclonal antibodies were tested a second time by DAC-ELISA for possible contamination with CPMoV, CPMV, CSMV, and SBMV.

Antisera to potyvirus isolate V17-14 were produced in two young laying chickens. A series of five intramuscular breast injections of 150–200 μg of purified potyvirus were made at weekly or biweekly intervals. Eggs were saved during a period of 10 wk, and IgG was extracted from the yolks of selected egg clutches by the methods of Jensenius et al (10). The chickens were anesthetized and exsanguinated 4 wk after the final injection. Blood- and yolk-derived IgGs were compared, and blood-derived IgG was chosen for DAS-ELISA tests of serological affinities among BlCMV, CABMV, and isolate V17-14.

Absorbance values were recorded by a Bio-Tek Model EL-309 ELISA reader, typically 90 min after addition of enzyme substrate (*p*-nitrophenyl phosphate). Tested antigens were buffered extracts

from fresh or desiccated tissues of virus-infected plants.

Electron microscopy. Preparations of leaf-dip extracts and partially purified virus were examined with a Philips EM 12 electron microscope. Preparations were adsorbed to carbon-coated copper grids and negatively stained with 2% ammonium molybdate (pH 7.0). Internal and external magnification standards were used for estimating virion sizes.

RESULTS

Field survey and virus detection. Of 66 samples collected from 37 locations among five cowpea growing areas of Senegal, 36 samples reacted positively with one or more of the seven test antisera or with potyvirus MAb II-197 (Table 1). Neither BlCMV, CMV, nor CPMV was detected among the Senegal test samples. Thirty-four of 66 samples (52%) contained CABMV. One sample (from Diourbel) contained both CSMV and CPMoV. One sample (from Louga) contained SBMV (in mixture with CPMoV). Twenty-one samples (32%) reacted with potyvirus MAb II-197, but did not react with any of the seven polyclonal antisera. Based on the exclusive reactions of these isolates with potyvirus-selective MAb and supplementary DAS-ELISA with known potyviruses, isolates V1-1 and V17-14 were concluded to be isolates of an unknown potyvirus, hereafter designated PTY+. The unknown potyvirus and CABMV were the predominant cowpea viruses at all locations surveyed in Senegal.

Inoculations and seed transmission. Disease incidence among five Senegal cultivars/lines mechanically inoculated with four PTY+ isolates (screenhouse and field) ranged from 8 to 100% (Table 2). Disease incidence was similar in inoculated screenhouse and field plantings.

Seed-transmission rates of the four potyvirus isolates in five Senegal cowpea genotypes varied from 0 to 30% (Table 2). The highest rate occurred with isolate V-17 in cultivar 58-57 which appeared to be most seed-transmission prone, whereas no seed transmission occurred in line IS86-283-15.

Preliminary host-range tests. Few host-range or host-reaction differences were found among the five seedborne potyvirus isolates tested (Table 3). Minor variations were observed in *Chenopodium amaranticolor* Coste & Reyn. and asymptomatic infection in *Phaseolus vulgaris* L. cv. Topcrop.

Screening cowpea cultivars for resistance. Reactions of cowpea lines/cultivars to mechanical inoculation with five seedborne PTY+ isolates were determined (Table 4). Five International Institute of Tropical Agriculture (IITA) TVU lines (TVU-401, TVU-408P2, TVU-1000, TVU-1016-1, and TVU-1582) and one U.S. cultivar, White Acre-BVR, were immune (i.e., asymptomatic and free of ELISA MAb-detectable virus) to all isolates. Some genotypes were susceptible to all PTY+ isolates. Other genotypes were susceptible to specific isolates; for example, Serido was susceptible only to PTY+ V17-14 and TVU-410 only to PTY+ V54-23. In contrast, TVU-984 was resistant only to PTY+ V54-23.

Aphid transmission. Seedborne PTY+ isolates V1-1 and V17-14 were both efficiently transmitted nonpersistently by *A. craccivora.* Isolate V1-1 was trans-

Table 3. Reactions by selected plant species to inoculation with seedborne isolates of Senegal potyvirus PTY+

Species	Disease reaction to PTY+ isolates				
	V1-1	V17-2	V17-14	V54-3	V54-23
Legumes					
Lupinus albus cv. Astra	–[a]	–	–	–	–
Medicago sativa cv. DuPuits	–	–	–	–	–
Trifolium pratense cv. Kenland	–	–	–	–	–
Phaseolus vulgaris cv. Monroe	–	–	–	–	–
Phaseolus vulgaris cv. Topcrop	–	–	LI	–	–
Vicia faba cv. Hertz Freya	VN	VN	VN	VN	VN
Nonlegumes					
Chenopodium amaranticolor, Corvallis strain	LLn	LLn	LLN,VN	LLn	LLn,VN
Nicotiana benthamiana, ATCC	SM	SM	SM	SM	SM
Gomphrena globosa, A. F. Ross strain	–	–	–	–	–
Phlox drummondii cv. Tall Mixed Color	–	–	–	–	–
Lycopersicon esculentum cv. Marglobe	–	–	–	–	–
Petunia hybrida cv. King Henry	–	–	–	–	–
Antirrhinum majus cv. Mixed Colors	–	–	–	–	–

[a] –, No symptoms and no ELISA-detectable virus; LI, latent (asymptomatic), ELISA-detected infection; LLn, necrotic local lesions; VN, vein necrosis; and SM, systemic mosaic.

Table 4. Responses of selected cowpea genotypes to greenhouse mechanical inoculations with five seedborne isolates of Senegal potyvirus PTY+

Genotype	Isolate				
	V1-1	V17-2	V17-14	V54-3	V54-23
TVU-109P2[a]	–[b]	++	++	–	++
TVU-196	++	–	++	++	++
TVU-347	–	++	–	++	–
TVU-354	++	++	++	++	++
TVU-401	–	–	–	–	–
TVU-408P2	–	–	–	–	–
TVU-410	–	–	–	–	+
TVU-984	++	++	++	++	–
TVU-1000	–	–	–	–	–
TVU-1016-1	–	–	–	–	–
TVU-1582	–	–	–	–	–
TVU-2657	++	++	++	++	++
TVU-3433	++	++	++	++	++
IT 81D 1137	–	++	++	++	++
IT 86 27N	++	++	++	++	++
PI 25122	++	++	++	++	++
Bambey 21	LI	–	–	++	++
Serido	–	–	++	–	–
White Acre-BVR	–	–	–	–	–
California Blackeye No. 5	0	0	+	++	0
Snapper	0	0	+	++	0
Blue Goose	0	0	–	–	0
Corona	0	0	–	–	0
Mopod	0	0	–	–	0
Texas Cream No. 8	0	0	++	++	0
Texas Cream No. 40	0	0	–	++	0
UC Riverside No. 524B	0	0	0	–	–
Mississippi Purple	0	0	++	++	0
Mississippi Silver	0	0	++	++	0
Magnolia	0	0	++	–	0
Knuckle Purple Hull	0	0	–	–	0
Worthmore	0	0	++	++	0
Bettergreen	0	0	++	++	0
UC Riverside No. 7964	0	0	++	++	0

[a] TVU genotypes kindly provided by the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The U.S. cowpea cultivars were kindly provided by O. L. Chambliss (Auburn, AL) or A. E. Hall (Riverside, CA).

[b] Symbols are: –, no symptoms and no virus detectable by ELISA, (immune); +, mild systemic symptoms; ++, moderate systemic syptoms; LI, latent (asymptomatic), ELISA-detected infection (tolerant to infection); 0, not tested.

mitted to 42 of 61 test plants, and isolate V17-14 to 59 of 59 test plants.

Serological relationships. Seedborne isolates of potyvirus PTY+ were serologically unrelated to BlCMV, CABMV, or PSbMV by DAS-ELISA, and were free of ELISA-detectable CMV (Table 5). The same isolates reacted to varying degrees in DAC-ELISA with antisera to BlCMV, CABMV, PeMV, pea mosaic virus (PMV), PSbMV, and PStV, but not with antisera to CYVV or WLMV. The PTY+ isolates were verified to be free of ELISA-detectable CPMoV, CSMV, PStV, or SBMV. All PTY+ isolates reacted with MAb II-197. Reactions of isolate V1-1 were consistently weaker with both MAb PTY and II-197.

Immunoglobulin to PTY+ isolate V17-14 reacted indistinguishably with PTY+ isolates, but did not react with 10 selected BlCMV isolates or with nine of 11 selected CABMV isolates (Table 6).

Electron microscopy. Leaf dips and partially purified preparations of PTY+ isolates contained flexuous rod-shaped particles with a modal length (>100 particles) of approximately 725 nm.

DISCUSSION

Cowpea viruses are increasingly important in all cowpea growing areas of Senegal. The survey reported herein was prompted because new pathogen/pest-resistant breeding lines had been damaged by viral diseases. Moreover, seedborne viruses were designated priority pathogens in these studies, since they have historically inflicted heavy losses through unknowing establishment of seedborne field inoculum, followed by secondary spread by insect-vector species (8).

The present study of 66 selected cowpea samples with viruslike symptoms indicated the presence in Senegal of four recognized seedborne viruses, CABMV, CSMV, CPMoV, and SBMV, and an apparently new potyvirus. Previously, SBMV had been reported from the Casamance region of Senegal (7) and CSMV in cowpea germ plasm accessions from Senegal (8), while CPMoV had been reported only from Nigeria (1,18) and in Pakistan (2). The seedborne nature of CPMoV (1,18) and these recent detections suggest that the virus is now spreading through seeds to other parts of the world. However, CABMV and potyvirus PTY+ were the prevalent viruses, occurring in 83% of the 66 samples and accounting for 55 of the 57 samples in which viruses were ELISA-detected. Based on prior investigations (8), CMV and CPMV were expected to occur in Senegal-grown cowpeas, but neither was detected. Although CPMV is known to occur in cowpeas in many areas of West Africa, it also reportedly has not occurred over extensive cowpea-production areas, for example the northern guinea savanna of Nigeria (20). The limited number of specialized cowpea genotypes sampled for this study could have influenced our nondetection of CMV.

Generally, the experimental host ranges for PTY+ isolates were more narrow than those for typical isolates of CABMV (3,4). No attempt was made to classify PTY+ pathotypes using these

Table 5. Comparisons of eight recognized potyviruses with five seedborne Senegal cowpea potyvirus isolates and four other seedborne viruses by DAS- and DAC-ELISA of plant extracts

	A_{405} value[a]						
	Seedborne isolates of Senegal potyvirus PTY+						
Antiserum	V1-1	V17-2	V17-14	V54-3	V54-23	Homologous virus[b]	Healthy-plant extract
DAS-ELISA							
BlCMV[c]	0.00	0.01	0.01	0.00	0.01	0.95	0.01
CABMV	0.01	0.01	0.01	0.01	0.03	1.15	0.01
PSbMV	0.00	0.00	0.00	0.00	0.00	1.87	0.00
CMV	0.01	0.00	0.01	0.00	0.00	1.27	0.02
DAC-ELISA							
BlCMV	0.26	1.20	0.36	0.37	0.21	2.81	0.02
CABMV	1.68	1.65	1.51	1.30	1.41	2.48	0.02
CYVV	0.11	0.10	0.08	0.05	0.08	>3.00	0.01
PeMoV	1.63	1.38	2.03	1.27	1.58	>3.00	0.03
PStV	1.90	1.50	1.75	1.10	1.92	>3.00	0.02
PMV	1.86	0.54	0.23	0.78	0.51	>3.00	0.02
PSbMV	1.65	1.58	1.51	1.86	2.11	>3.00	0.01
WLMV	0.01	0.02	0.01	0.02	0.04	>3.00	0.01
CPMoV	0.03	0.02	0.02	0.02	0.00	>3.00	0.03
CSMV	0.16	0.10	0.11	0.15	0.13	>3.00	0.12
SBMV	0.00	0.00	0.00	0.00	0.02	>3.00	0.01
II-197							
BCMV MAb	0.31	0.77	1.40	0.57	0.70	0.58	0.00
Agdia							
PTY MAb	0.22	NT[d]	0.70	0.78	0.86	0.77	0.00

[a] A_{405} values recorded after 90 min incubation with substrate, *p*-nitrophenyl phosphate.
[b] Virus homologous to each antiserum. BlCMV isolate RF-26B was selected as a positive control for MAb II-197. CABMV isolate 9-7C was selected as a positive control for the Agdia PTY MAb.
[c] Antisera to potyviruses: BlCMV, blackeye cowpea mosaic virus; CABMV, cowpea aphid-borne mosaic virus; CYVV, clover yellow vein virus; PMV, pea mosaic virus; PeMoV, peanut mottle virus; PSbMV, pea seedborne mosaic virus; PStV, peanut stripe virus; and WLMV, white lupin mosaic virus. Antisera to other viruses seedborne in cowpea: CMV, cucumber mosaic virus; CSMV, cowpea severe mosaic virus; SBMV, southern bean mosaic virus; and CPMoV, cowpea mottle virus. The Agdia PTY monoclonal antibody reacts to >90% of all tested potyvirus; monoclonal antibody II-197, produced against bean common mosaic virus, reacts to its homologue and several other potyviruses.
[d] NT = Not tested.

Table 6. DAS-ELISA tests of selected isolates of blackeye cowpea mosaic (BlCMV) and cowpea aphid-borne mosaic (CABMV) potyviruses against chicken anti-V17-14 immunoglobin G

Virus isolate[a]	A_{405} value
BlCMV	
BlCMV-Ga	0.003
PI-3B	0.005
RF-4B	0.012
PU-7B	0.004
PU-8B	0.003
PI-22B	0.007
PI-23B	0.002
PI-25B	0.003
RF-26B	0.002
RF-27B	0.007
CABMV	
RN-7C	0.004
RN-10C	0.003
RN-27C	0.934
RN-28C	0.669
RN-34C	0.012
RN-35C	0.004
RN-36C	0.002
RN-37C	0.002
PI-39C	0.005
PI-40C	0.002
PI-44C6	0.001
V17-14 (homologue)	0.758
Healthy-plant extract	0.013

[a] Isolates of BlCMV and CABMV were each seedborne in cowpea and isolated from infected seedlings, except for BlCMV RF- isolates which originated in naturally infected cowpea seed fields in the San Joaquin Valley of California. The other isolate prefixes are: PI = isolates derived from seedlings of selected U.S. *Vigna unguiculata* germ plasm accessions; RN = isolates from young seedlings in a USDA-funded *V. unguiculata* preintroduction germ plasm nursery; PU = isolates derived from seedlings of an India cowpea cv. Pusa Phalgoni. Isolates BlCMV-Ga (Georgia) and RN-7C (Botswana) were included as type isolates of BlCMV and CABMV, respectively. Isolates RN-27C and RN-28C previously had reacted in DAS-ELISA only to CABMV antiserum produced against CABMV isolate RN-7C.

cowpea genotypes; however, the genotypes provided evidence that the isolates were pathogenically diverse.

Despite clear serological distinctions among PTY+, BlCMV, and CABMV, the response of cowpea genotypes to PTY+ inoculation resembled those to CABMV, especially the resistance of IITA accessions TVU-401 and TVU-1582, previously classified as resistant to all tested CABMV isolates (3,4). The PTY+ isolates may represent a distinct serotype and pathotype of CABMV. In any case, the five CABMV-resistant Senegal cultivars herein studied are susceptible to PTY+, whereas TVU-401 and TVU-1582 appear to contain multiple genes/alleles conferring resistance to both PTY+ and all tested CABMV variants (3,4).

Cowpea line IS86-283-15 was partially resistant or tolerant to two of four PTY+ field isolates, V-1 and V-2. Larger numbers of seeds are required than herein tested to assess real differences in seed-transmission rates among PTY+ isolates in Senegal cultivars selected for this study.

Multiple-virus infections are common among samples from field-grown cowpeas, worldwide, and are known to modify and complicate symptoms, thus precluding in-field diagnosis based on symptomatology (9,12). In our study, however, mixtures of seedborne viruses were found in only two of the 66 cowpea tissue samples (CSMV + CPMoV and SBMV + CPMoV). Limited genotype sampling also could have influenced this result.

We believe that *A. craccivora* is a principal vector of all seedborne potyviruses in Senegal and that indigenous biotypes can transmit isolates of potyvirus PTY+ at rates comparable to those obtained in our study.

Differences in relative affinities between PTY+ isolates and known potyviruses when compared by DAS- vs. DAC-ELISA are understandable. Use of DAC-ELISA is generally acknowledged to result in fewer antigen-discriminating assays than DAS-ELISA.

Reactions to PTY+ antiserum by CABMV isolates RN-27C and RN-28C were unexpected, since the isolates were previously considered typical, pure CABMV isolates. We did not determine whether the results indicated the sharing of coat protein epitope(s) between RN isolates and PTY+ V17-14 (i.e., all three comprising a distinct CABMV serotype) or possible contamination of RN isolates with PTY+. Both RN isolates had originated in cowpea seeds obtained from Botswana (3,4).

The estimated 725-nm modal length of particles associated with PTY+ fits within the recognized 710–900 nm size range of potyvirus particles.

While disease-control measures, including control of insect vectors, removing diseased plants from seed fields, and production of virus-free seed, may impede the progress of cowpea viral diseases, we believe that development of resistant cultivars is the most practical and economical control measure for these diseases. In this study, we identified six cowpea genotypes as sources of resistance to all PTY+ isolates. These genotypes have been incorporated into the extant Senegal cowpea breeding program for development of improved disease/pest-resistant cultivars for Senegal cowpea production areas.

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