Partial Characterization and Grouping of Isolates of Blackeye Cowpea Mosaic and Cowpea Aphidborne Mosaic Viruses

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ABSTRACT

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The biological, physical, and some biochemical properties of single isolates of blackeye cowpea mosaic virus (BlCMV) from Florida and New York were compared with those of single isolates of cowpea aphidborne mosaic virus (CAMV) from Cyprus, Morocco, Kenya, and Nigeria. On the basis of reactions on selected cowpea lines, the six isolates were classified into the BlCMV or CAMV group. No common source of resistance was found for both CAMV and BlCMV, but cowpea lines with resistance to each virus group were identified. These resistant lines were used for differentiating the isolates as BlCMV or CAMV. The BlCMV group consisted of the Florida and New York isolates of BlCMV and the Kenya and Nigeria isolates of CAMV, whereas the CAMV group included the Morocco and Cyprus isolates. In reciprocal cross-protection tests, neither the Kenya nor Florida isolates cross-protected against the Morocco isolate, and vice versa. However, the size of the capsid protein, the sedimentation rate of the nucleic acid, and the length of the particles of the six isolates were very similar. The impact of this regrouping on the taxonomic status of BlCMV and CAMV is discussed.

Blackeye cowpea mosaic virus (BlCMV) and cowpea aphidborne mosaic virus (CAMV) are two potyviruses pathogenic to cowpea (eg, 33). BlCMV was first reported in the United States by Anderson (2). By microagglutination tests, Corbett (12) concluded that a virus disease of cowpea *Marmor vignae* was caused by a strain of bean yellow mosaic virus (BYMV); as a result of this observation, several authors have referred to BlCMV as the cowpea strain of BYMV (19,23,24,30,31). However, Edwardson et al (14), in a comparative study of cytological inclusions induced by BYMV and BlCMV, concluded that BYMV and BlCMV should be considered as distinct members of the potyvirus group. This conclusion was further supported by the host-range studies of Zettler and Evans (54).

In 1966, Lovisolo and Conti (34) described a flexuous rod-shaped, aphidborne and seedborne virus affecting cowpea in Italy and designated it cowpea aphidborne mosaic virus (CAMV). Other workers have reported viral isolates having properties similar to those of CAMV from several parts of the world (1,4,5,9,15, 28,32,40,41,45,48).

Recently, Lima et al (33) compared an isolate of BlCMV with an unusual strain of CAMV from Morocco (15). By host range and nonreciprocal sodium dodecyl sulfate (SDS)-immunodiffusion tests with antiserum to BlCMV, they concluded that BlCMV and CAMV were distinct potyviruses. However, some workers (6,37) consider BlCMV and CAMV to be closely related or synonymous.

BlCMV has only been reported in the United States, while CAMV occurs in other parts of the world. We suspected a misidentification of some of the CAMV isolates due to a lack of direct comparison with BlCMV. A correct identification and classification of isolates of CAMV and BlCMV is considered essential for effective control with resistant cultivars and to define the actual geographical distribution of BlCMV and CAMV. This study was, therefore, conducted to provide more information about the identity and properties of some CAMV isolates from Kenya, Nigeria, and Cyprus by comparing them directly with isolates of BlCMV from New York (46) and Florida (33), and an isolate of CAMV from Morocco (15).

Our results show that although CAMV and BlCMV have similar physical properties, they can be grouped according to differences in host range on cowpea lines. Moreover, two isolates previously regarded as CAMV were identical to BlCMV. Immunological characterizations of these six isolates (44) are in agreement with this proposed grouping.

MATERIALS AND METHODS

Source and maintenance of virus cultures. BlCMV isolates were obtained from Florida (D. Purcifull) and New York (J. K. Uyemoto) and were designated BlCMV-Fla2 and -Flo, respectively. Cowpea aphidborne mosaic virus (CAMV) isolates were from Cyprus (R. Provvidenti), Kenya (K. Bock), Morocco (A. Lima and D. Purcifull, a subculture of an isolate originally obtained from B. Lockhart and H. Fischer), and Nigeria (H. W. Rossel) and were designated CAMV-Cyp, -Ken, -Mor, and -Nig, respectively. After three successive single-lesion transfers on *Chenopodium quinoa* Willd, stock cultures of each isolate were propagated in California Blackeye cowpea plants and transferred every 4–6 wk by mechanical inoculation.

Tobacco mosaic virus (TMV) strain U_1 isolated (M. Zaitlin) from and propagated in Turkish tobacco, whereas bromegrass mosaic virus (BMV) was obtained from E. Hiebert and maintained on barley.

Virus purification. All isolates were purified from blackeye peas 18–24 days after inoculation. The Flo and Fla2 isolates of BlCMV and the Nig, Ken, and Mor isolates of CAMV were purified by the procedure of Lima et al (33) with the following modifications. Ethylenediamine tetracetic acid (EDTA, 0.01 M) was used in the grinding buffer and two cycles of CsCl density gradient centrifugation were used instead of one. After the first CsCl centrifugation, the viral zone was removed, diluted with two volumes of PM buffer (0.02 M potassium phosphate, pH 8.2 + 0.1% 2-mercaptoethanol), and centrifuged at 10,000 rpm for 10 min in a Sorvall SS 34 rotor. The supernatant fluid was made to 30% (w/w) CsCl and recentrifuged in a Beckman 40 rotor at 29,000 rpm for 20–24 hr. The Cyp isolate was purified by a similar procedure, but centrifugation was in Cs_2SO_4 instead of CsCl. Cs_2SO_4 (0.15 g/ml) was dissolved in the virus suspension, then layered (10 ml per tube) over a 3.0-ml cushion of 53% (w/w)

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Cs_2SO_4 in PM buffer. Yield of purified virus was estimated assuming an $E^{0.1\%}_{260\ nm} = 2.4$ (42) uncorrected for light scattering.

Polyacrylamide gel electrophoresis of viral coat protein. Polyacrylamide gel electrophoresis (PAGE) of SDS-dissociated capsid protein was performed by using the method of Weber and Osborn (49), except that the molarity of the electrophoresis buffer was reduced by half (25). Purified virus was resuspended in 0.01 M sodium phosphate, pH 7.0, containing 1% SDS and 1% 2-mercaptoethanol, heated in boiling water for 1 min, and stored at −10 C. Just prior to electrophoresis, the dissociated virus preparation (30–50 μg/gel) was reheated in boiling water and adjusted to 10% (w/v) with sucrose. The molecular weight (MW) of the capsid protein of all BlCMV and CAMV isolates was estimated in 5%, 7.5%, and 10% (w/v) polyacrylamide tube gels. Bovine serum albumin (MW 68,000), glutamate dehydrogenase (MW 53,000), carbonic anhydrase (MW 29,000), and chymotrypsinogen (MW 25,700) were used as protein markers. The line of best fit for the markers was determined for each experiment by regressing the logarithms of their MW against their relative mobilities.

TMV was purified by the method of Whitfield and Williams (52), and BMV was purified by a method outlined by E. Hiebert (*personal communication*).

Preparation and sedimentation rate of viral nucleic acid. BlCMV and CAMV RNAs were prepared by a method similar to that of Brakke and Van Pelt (8). Virus obtained after the first or second cycle of purification in CsCl or Cs_2SO_4 was precipitated with 8% PEG and resuspended in distilled water. The suspension was mixed with an equal volume of a dissociation buffer consisting of 0.2 M ammonium carbonate, 0.002 M EDTA, 2% SDS, and 0.2% sodium diethyldithiocarbamate, pH 9.0, containing 100–400 μg of EDTA-treated bentonite per milliliter (16). Proteinase K was added at 10 μg/ml (13), the mixture was left at room temperature for 20 min, and then layered on preformed linear-log sucrose density gradients (7) in 1× SSC (0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0). Centrifugation was at 23,000 rpm in Spinco SW 25.1 rotor for 16.5 hr at 13 C. UV-absorbing zones were detected and collected using an ISCO UA-5 density gradient scanner and 640 fractionator (Instrumentation Specialties Co., Lincoln, NB 68504). The viral RNA was precipitated with ethanol, stored at −10 C overnight, centrifuged at 8,000 rpm for 10 min in a Sorvall SS 34 rotor, and resuspended in 1× SSC. The concentration of the RNA was determined spectrophotometrically using $E^{0.1\%}_{260\ nm} = 25$. RNA either was used immediately or stored at −10 C.

The infectivity of the RNA was determined by adding 100 μg of bentonite to 10 μg of RNA in 1 ml of 1× SSC and inoculating *C. quinoa* with sterile cotton swabs. Lesions were counted after 10 days.

The RNAs of TMV and BMV were extracted by dissociating ~30 mg of freshly purified virus in an equal volume of the dissociation buffer of Brakke and Van Pelt (8), and in the presence of 78% phenol containing 0.1% 8-hydroxyguinoline and 10% *m*-cresol (21). The RNAs were precipitated and resuspended in 1× SSC as above.

Sedimentation coefficient values were estimated by the method of Brakke and Van Pelt (7). The RNAs of TMV and BMV were used as markers.

Host range and screening of cowpea lines for sources of resistance. Plants of each host were inoculated with sap extracted from California Blackeye peas individually infected with CAMV-Mor, -Cyp, -Ken, -Nig, BlCMV-Fla2, or -Flo, by using P buffer (0.05 M potassium phosphate, pH 7.5) as extraction buffer. Four weeks after inoculation, both the inoculated primary leaves and the trifoliolate leaves were assayed for BlCMV or CAMV by the enzyme-linked immunosorbent assay (ELISA) as outlined by Clark and Adams (11), and modified by Taiwo and Gonsalves (44). Several cowpea lines obtained from the Grain Legume Programme, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, were included in this study.

Stability of virus in sap. Thermal inactivation point (TIP), longevity in vitro (LIV), and dilution end point (DEP) were determined for BlCMV-Fla2, CAMV-Mor, and -Ken by using *C. quinoa* as the assay host. All test plants were observed for 3 wk for chlorotic local lesions.

Virus particle-size determination. Leaf-dip preparations from systemically infected cowpea plants were negatively stained in 2% potassium phosphotungstate, pH 7.2, containing 0.1% bovine serum albumin. The virus particles were observed with a JEOL JEM 100B electron microscope. From 45 to 100 particles were measured for each of the six isolates. The particle sizes were estimated by comparing their projected micrographs with that of a diffraction grating at the same magnification.

Cross-protection. Initial tests showed that CAMV or BlCMV could be detected in trifoliolate leaves of cowpea 7 days after the primary leaves had been inoculated. Cross-protection was, therefore, investigated in cowpeas by inoculating primary leaves with BlCMV-Fla2 or CAMV-Mor (designated primary inoculation), followed by subsequent inoculation (challenge inoculation) of the trifoliolate leaves with the challenge virus 10, 14, and 21 days later. Healthy cowpea plants of comparable age were inoculated with the challenging virus at the time of challenge inoculation. Inoculum for primary and challenge inoculations were prepared from tissue extracts obtained from comparably infected cowpea leaves by using P buffer. After challenge inoculation, plants were left for 15 days, then the virus content in systemically infected leaves was estimated by ELISA (11,44) utilizing antisera to BlCMV-Fla2 and CAMV-Mor. The virus concentration was calculated from the ELISA absorbance values by a linear regression equation.

RESULTS

Virus purification. Both isolates of BlCMV and the Ken, Mor, and Nig isolates of CAMV were purified in CsCl. CAMV-Cyp did not band in CsCl, but did band in Cs_2SO_4. Most virus preparations

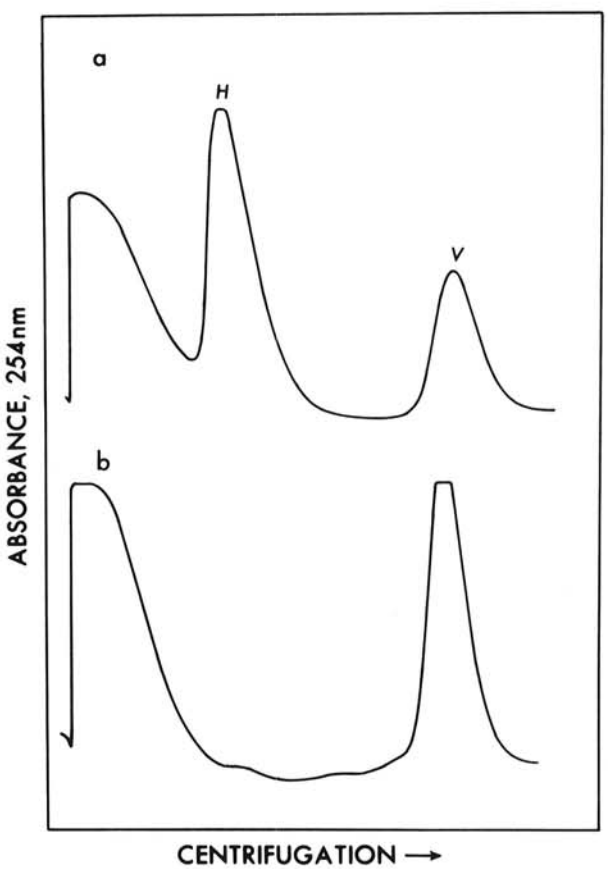

Fig. 1. Ultraviolet absorbency profiles of viral RNA centrifuged in linear-log sucrose density gradients prepared in 1×SSC, pH 7.0. Centrifugation was at 13 C and 23,000 rpm for 16.5 hr in a SW 25.1 rotor. Profile of RNA of blackeye cowpea mosaic virus (BlCMV) obtained from dissociation of purified virus preparation after one cycle (curve a) and two cycles (curve b) of CsCl density gradient centrifugation. V denotes viral RNA, and H denotes the presumed host DNA.

TABLE 1. Effect of EDTA on yield of purified preparations of blackeye cowpea mosaic virus (BlCMV) and cowpea aphidborne mosaic virus (CAMV)

Virus isolate[a]	EDTA molarity[b] 0	0.001	0.01	0.1
Fla2	0.60[c]	0.80	1.50	1.45
Flo	4.25	4.10	7.70	5.00
Ken	3.90	2.00	5.40	3.90
Mor	1.01	2.20	3.00	2.20
Cyp	3.09	3.15	4.70	4.50
Nig	1.45	2.97	3.70	2.50

[a] Fla2 and Flo are isolates of BlCMV from Florida and New York. Ken, Mor, Cyp, and Nig are isolates of (CAMV) from Kenya, Morocco, Cyprus and Nigeria, respectively.
[b] Molarity of EDTA in grinding buffer.
[c] Yield (mg per 100 g tissue) are means of two or three experiments.

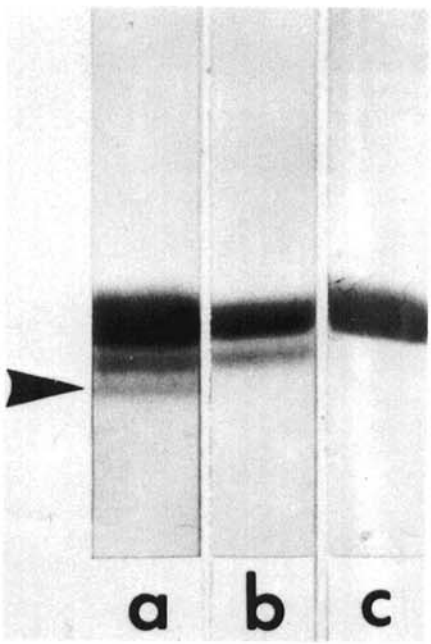

Fig. 2. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of blackeye cowpea mosaic virus (BlCMV) and cowpea aphidborne mosaic virus (CAMV) capsid protein; **a,** BlCMV-Fla2, **b,** BlCMV-Flo, and **c,** CAMV-Ken. Purified virus preparations were given two cycles of centrifugation in CsCl and were dissociated after **a,** 20 days, or **b and c,** immediately after purification. Arrow denotes the location of carbonic anhydrase (MW 29,000), the marker protein. Electrophoresis was from top to bottom in 7.5% gel. Molecular weight estimates were: **a,** 35,000, 31,000, and 29,000; **b,** 35,000 and 31,000; and **c,** 34,500.

after one density gradient cycle were contaminated with what appeared to be host DNA (Fig. 1a), which has been reported by others (8,13,35). A second density gradient cycle eliminated the DNA (Fig. 1b). Virus yield after two cycles was about 2.5 mg per 100 g of tissue.

EDTA in the grinding buffer appeared to increase virus yield (Table 1). Although there were no significant differences between the treatments, 0.01 M EDTA consistently gave higher virus yields and was used thereafter.

Virus protein. PAGE of viral coat protein revealed one to three bands with the largest proportion being in the slowest migrating form (Fig. 2a–c). The MW for both isolates of BlCMV and the four isolates of CAMV were 34,000–35,000 for the slowest, 31,000 for the intermediate, and 29,000 for the fastest band. The estimated MW were not independent of the acrylamide concentration of the gels (Table 2).

Virus preparations partially purified by a single cycle of centrifugation on CsCl or Cs_2SO_4 before storage at 4 C for >1 mo contained only the fastest band (Fig. 3a and b). Aliquots of those virus preparations given two Cs_2SO_4 cycles before storage at 4 C did not show appreciable conversion into the fast form (Fig. 3c and d).

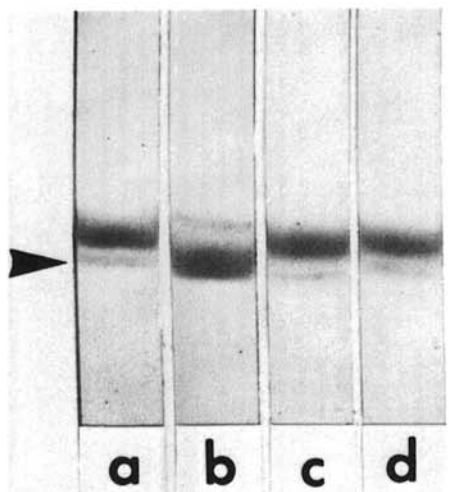

Fig. 3. Polyacrylamide gel electrophoresis to determine the effect of purification procedure and storage at 4 C on degradation of capsid protein of the Cyprus isolate of cowpea aphidborne mosaic virus (CAMV-Cyp). Virus partially purified by one Cs_2SO_4 centrifugation cycle and analyzed **a,** immediately or **b,** 32 days later. Virus purified by two Cs_2SO_4 centrifugation cycles and analyzed **c,** immediately or **d,** 32 days later. Arrow denotes the location of carbonic anhydrase (MW 29,000), one of the marker proteins. Electrophoresis was from top to bottom in 10% gels. Molecular weight estimates were 35,000 and 30,000.

TABLE 2. Molecular weight estimates of viral coat protein of isolates of blackeye cowpea mosaic virus (BlCMV) and cowpea aphidborne mosaic virus (CAMV) by sodium dodecyl sulphate polyacrylamide gel electrophoresis

	Molecular weight estimates[a] of isolates[b]												
	Fla2			Flo		Ken		Nig		Mor		Cyp	
Gel (%)	Slow[c]	Int	Fast	Slow	Fast	Slow	–	Slow	Fast	Slow	Fast	Slow	Fast
5.0	37,311 ±1,146	···	33,252 ±464	36,653 ±884	33,199 ±1,142	36,094 ±464		37,386 ±1,139	32,453 ±836	34,032 ±808	30,430 ±724	35,924 ±321	32,050 ±213
7.5	34,996 ±640	31,845 ±833	29,432 ±493	34,937 ±657	30,572 ±628	34,423 ±356		34,601 ±886	28,773 ±1,068	33,860 ±1,099	29,710 ±365	33,688 ±1,095	29,659 ±1,471
10.0	34,620 ±797		31,821 ±913	34,488 ±487	30,871 ±143	34,072 ±964		34,084 ±1,010	30,066 ±1,136	32,028 ±695	28,650 ±722	33,810 ±588	31,038 ±819

[a] Molecular weight estimates with the standard deviation are the means of three to six experiments for each type of acrylamide gel concentration, using one or two different purified preparations for each isolate.
[b] Single isolates of BlCMV from Florida (Fla2) and New York (Flo), and single isolates of CAMV from Kenya (Ken), Nigeria (Nig), Morocco (Mor), and Cyprus (Cyp).
[c] Slow, intermediate (Int), and fast refer to the top, middle, and bottom protein bands in polyacrylamide gels.

Isolation, infectivity, and sedimentation velocity of the RNA of BlCMV and CAMV. Linear-log density gradient centrifugation of dissociated purified virus revealed two ultraviolet (UV)-absorbing zones when purified virus was given only one CsCl or Cs_2SO_4 cycle of centrifugation (Fig. 1a) before it was dissociated. Further purification of such preparations in CsCl or Cs_2SO_4 resulted in virtual elimination of the slower sedimenting peak (Fig. 1b). Recovery of RNA was about 65%.

The average sedimentation coefficient for the RNAs and the standard deviation for three experiments were: BlCMV-Fla2, 38.9 ± 0.7; -Flo, 41.5 ± 0.7; CAMV-Ken, 39.7 ± 0.9; -Nig, 39.6 ± 0.5; -Mor, 40.6 ± 0.7; and -Cyp, 39.4 ± 0.5. No difference in the sedimentation behavior of BlCMV-Fla2 and CAMV-Mor2 RNAs was detected (Fig. 4).

The infectivity of the viral RNAs (10 μg/ml) from five of the six isolates were tested on *C. quinoa*. The average number of lesions per leaf from two experiments varied from 17 for BlCMV-Flo to 38 for CAMV-Mor. RNA from CAMV-Ken produced 25 lesions, whereas CAMV-Cyp and BlCMV-Fla2 produced 28 and 23 lesions, respectively. Inoculation with purified virus (10 μg/ml) resulted in 24, 44, 39, 30, and 31 lesions per leaf for BlCMV-Flo, CAMV-Mor, -Ken, -Cyp, and BlCMV-Fla2, respectively.

Host range and sources of resistance. BlCMV and CAMV were readily transmitted mechanically from California Blackeye to several cowpea cultivars obtained from D. Purcifull and lines obtained from IITA. No single cowpea line was immune to both CAMV and BlCMV (Table 3), but some bean cultivars (Black Turtle Soup 1 and Great Northern 1140) were resistant to both viruses.

Isolates of BlCMV-Fla2, -Flo, CAMV-Ken, and -Nig had nearly identical host ranges (Table 3). There were, however, slight differences in symptom severity. BlCMV-Flo and CAMV-Ken produced similar but more severe symptoms than BlCMV-Fla2 and CAMV-Nig on California Blackeye cowpea. Symptoms induced by BlCMV-Fla2 and CAMV-Nig on this host were similar.

The Mor and Cyp isolates of CAMV induced similar symptoms and had fairly similar host ranges, but those of CAMV-Mor and -Cyp differed widely from those of CAMV-Ken and -Nig (Table 3).

Of 58 cowpea cultivars and lines tested, five lines had plants that were resistant to CAMV-Mor and -Cyp (Table 3). For example, five of six plants from line 196 were resistant to CAMV-Mor, whereas all the five plants inoculated with CAMV-Cyp were

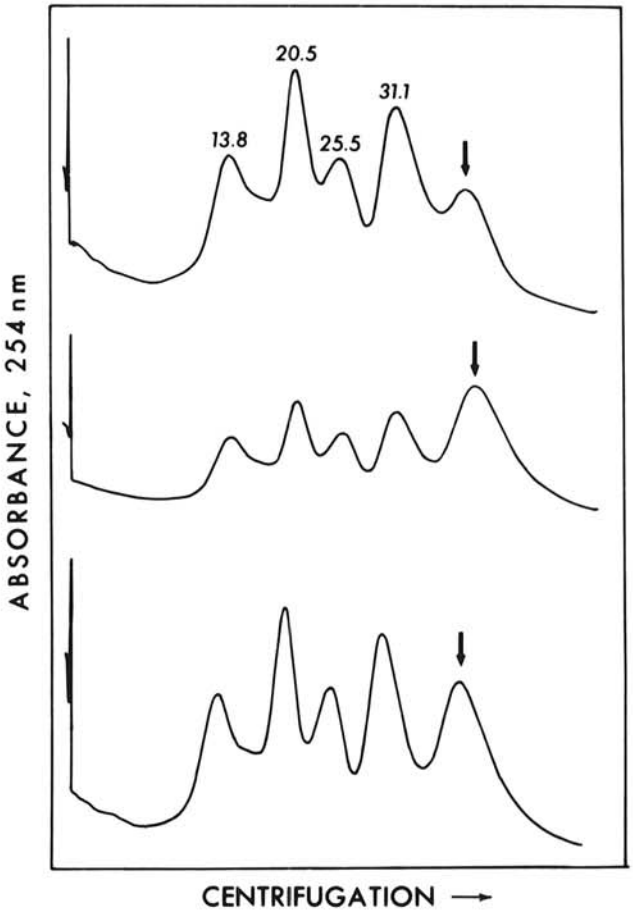

Fig. 4. Ultraviolet absorbency profiles of viral RNA species centrifuged in linear-log sucrose density gradients prepared in 1 × SSC, pH 7.0. Centrifugation was at 13 C and 23,000 rpm for 16.5 hr in SW 25.1 rotor. Standard RNAs are brome mosaic virus (13.8, 20.5, and 25.5 S species) and tobacco mosaic virus (31.1 S). The arrows denote the RNAs of (top) blackeye cowpea mosaic virus (BlCMV-Fla2), (middle) cowpea aphidborne mosaic virus (CAMV-Mor), and (bottom) a mixture of BlCMV-Fla2 and CAMV-Mor.

TABLE 3. Reaction of *Vigna unguiculata* to mechanical inoculation with isolates of blackeye cowpea mosaic virus (BlCMV) and cowpea aphidborne mosaic virus (CAMV)

Host	Isolates[a]					
	Fla2	Flo	Ken	Nig	Mor	Cyp
California Blackeye	GVB[b]	GVB-S	GVB-S	GVB	CD,B,GVB-S	CD,B,GVB-S
Bola de Ouro[c]	–	–	–	–	Mt	Mt
CE 73	Mt	Mt	Mt	Mt	Mt	Mt
Serido	–	–	–	–	Mt	Mt
Sete Semanas	–	–	–	–	Mt	Mt
Crowder pea	–	–	–	–	Mt	Mt
IITA TVU[d]						
2480	–	–	–	–	VC*	VC*
2657	–	–	–	–	B-Mt,CD	B-Mt,CD
2740	–	–	–	–	B-Mt,CD	B-Mt,CD
2845	–	–	–	–	B-Mt,CD	B-Mt,CD
3273	–	–	–	–	VP,B-Mt	VP,B-Mt
3433	–	–	–	–	VP,B-Mt	VP,B-Mt
196	Mt	B-Mt	B-Mt	Mt	1/6[e]	0/5
401	Mt	Mt	B-Mt	Mt	2/6	2/7
1582	Mt,GVB	Mt,GVB	Mt,GVB	Mt,GVB	0/5	0/3
1593	Mt	Mt	Mt	Mt	3/7	0/8
2460	Mt,GVB	Mt,GVB	Mt,GVB	Mt,GVB	3/9	1/10

[a] Florida (Fla2) and New York (Flo) isolates of BlCMV. Kenya (Ken), Nigeria (Nig), Morocco (Mor), and Cyprus (Cyp) isolates of CAMV.

[b] Abbreviations for symptoms: B = blister; B-Mt = bright yellow mottle; CD = downward cupping and deformation; GVB = green vein banding with normal sized leaves; GVB-S = green vein banding with reduction in leaf size; Mt = mottle; – = no symptoms; VC* = veinal chlorosis, sometimes symptomless; VP = veinal prominence.

[c] Cowpea cultivars used by Lima et al (33) obtained from D. Purcifull.

[d] Cowpea lines obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria.

[e] Number of plants susceptible (numerator) out of total tested (denominator). Eight to 10 plants were used in all other tests.

resistant (Table 3). In limited tests, one line was resistant to both isolates.

The reactions of two differential cowpea lines to inoculations with the six isolates are shown in Fig. 5. Virus infection in plants used for host range studies was confirmed by ELISA.

Stability of virus in sap. In undiluted sap from infected plants, BlCMV-Fla2, CAMV-Mor, and -Ken had a TIP between 57 and 60 C; LIV at 22 C was between 24–48 hr; and DEP between 10^{-4} and 10^{-5}.

Particle sizes. Flexuous, rod-shaped particles were consistently found in leaf-dip preparations from cowpea leaves individually infected with BlCMV-Fla2, -Flo, CAMV-Ken, -Nig, -Mor, and -Cyp. Of the particles measured for each isolate, 60 to 81% were between 720 and 760 nm, with a mode at 743 nm.

Cross-protection. Standard curves of virus concentrations versus absorbance values obtained in ELISA tests were generated for BlCMV-Fla2 and CAMV-Mor. Figure 6 shows the curve for BlCMV-Fla2. The regression of absorbance against concentration was $Y = -117.7 + 183.8\ X$ for BlCMV-Fla2 with a correlation coefficient (r) of 0.97, and $Y = -91.4 + 592.4\ X$ with $r = 0.98$ for CAMV-Mor. These equations were used to estimate the concentration of antigenic protein in 0.15 g of tissue from plants receiving the different treatments shown in Table 4.

These data do not suggest any cross-protection between BlCMV-Fla2 and CAMV-Mor (Table 4). However, concentrations of the challenging virus were not as high as in the controls. Similar trends (*unpublished*) were observed when the experiment was repeated, and when CAMV-Ken was used instead of BlCMV-Fla2. In plants simultaneously inoculated with both BlCMV-Fla2 and CAMV-Mor, CAMV-Mor developed a higher concentration of virus (Table 4). No drastic changes in symptomatology or plant growth were observed when plants were challenge-inoculated with either of the viruses. Symptoms induced were mostly those caused by the primary virus.

DISCUSSION

The classification of BlCMV and CAMV was in a state of confusion until recently, when Lima et al (33) suggested BlCMV and CAMV should be regarded as distinct members of the potyvirus group. Their suggestion was based on differences in host range and a distant serological relationship between one isolate of CAMV from Morocco and BlCMV. This suggestion has been accepted by the International Committee on the Taxonomy of

TABLE 4. The estimated concentration of the Florida isolate of blackeye cowpea mosaic virus (BlCMV-Fla2) and the Morocco isolate of cowpea aphidborne mosaic virus (CAMV-Mor) in California Blackeye peas used to investigate the cross-protection phenomenon

Days between primary and challenge inoc.	Challenging virus	Virus concentration[a] (μg/0.15 g of tissue)				Ratio of virus in mixture to control:	
		In mixture		In control			
		Mor	Fla2	Mor	Fla2	Mor	Fla2
0	–	17.2	2.5	18.3	11.6	0.94	0.22
	Mor	6.6	3.0	10.4		0.63	
10	Fla2	8.8	0.6		4.6		0.13
	Mor	5.5	8.2	15.6		0.35	
14	Fla2	11.0	3.6		9.2		0.39
	Mor	10.6	5.8	15.1		0.70	
21	Fla2	10.9	0.9		10.0		0.09

[a] Virus concentration was estimated by enzyme-linked immunosorbent assay, and by substituting the absorbance values for X in the following equations: $Y = -117.7 + 183.8\ X$ for BlCMV-Fla2 and $Y = -91.4 + 592.4\ X$ for CAMV-Mor, Fig. 6.

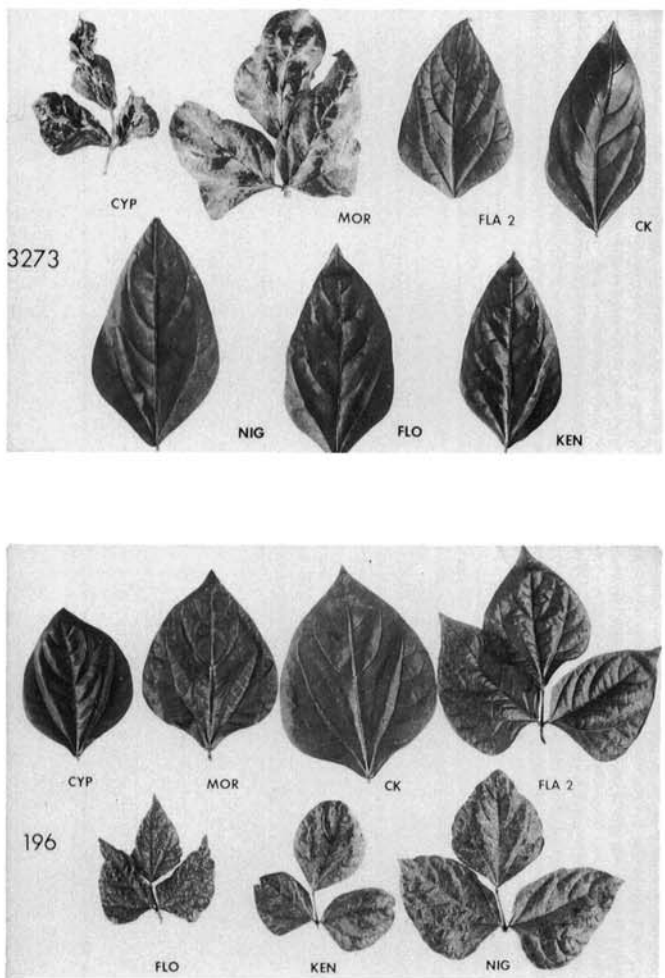

Fig. 5. Reaction of diagnostic cowpea lines to blackeye cowpea mosaic virus (BlCMV) and cowpea aphidborne mosaic virus (CAMV). Cowpea lines 3273 and 196 were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. FLA2, FLO, KEN, NIG, MOR, and CYP represent leaves taken from cowpea plants inoculated with the Florida and New York isolates of BlCMV and the Kenya, Nigeria, Morocco, and Cyprus isolates of CAMV, respectively. CK represents leaf from a plant inoculated with buffer.

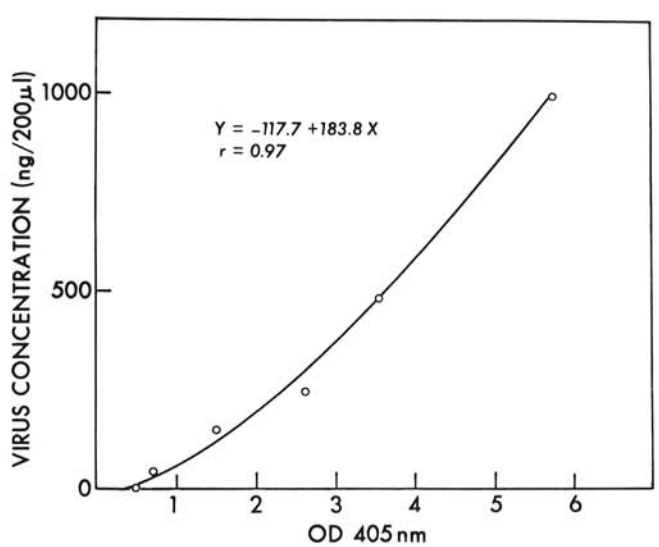

Fig. 6. Plot of virus concentration against absorbance. Healthy cowpea tissue (0.15 g) was ground in 7.5 ml of 0.1 M potassium phosphate plus 0.1 M EDTA, pH 7.5, and purified virus was added to give the desired concentration. Enzyme-linked immunosorbent assay was performed according to the procedure of Clark and Adams (11). A linear regression equation and correlation coefficient are shown for the Florida isolate of blackeye cowpea mosaic virus (BlCMV-Fla2). A similar curve was generated for the Morocco isolate of cowpea aphidborne mosaic virus.

Viruses who now consider CAMV a member and BlCMV a possible member of the potyvirus group (39). Our comparison of two isolates of BlCMV and four isolates of CAMV further justify this separation. Different genetic factors in cowpeas apparently confer resistance to CAMV and BlCMV in this host and immunological characterization of these isolates has shown that BlCMV and CAMV are serologically distinct (44). The two BlCMV isolates, CAMV-Ken, and -Nig are serologically unrelated to CAMV-Mor and -Cyp, whereas CAMV-Mor and -Cyp are distantly related to the other four isolates (44). Based on host range and serological similarities, the Kenya and Nigeria isolates should be regarded as BlCMV isolates instead of CAMV.

The cross-protection or interference phenomenon occurs between many virus strains and is often used to establish relatedness and, at times, as a criterion for classifying viruses (22,29,38). In general, unrelated viruses do not show cross-protection. The results of two separate experiments do not suggest BlCMV and CAMV cross-protect each other in California Blackeye peas. However, we do not regard cross-protection as a strong criterion for determining relatedness between viruses since some otherwise closely related viral isolates do not cross-protect (10,18,53), whereas some unrelated viruses show unilateral protection (17). However, partial interference in the production of antigenic coat protein was observed for both viruses, when either was present in the host before the other was introduced. This type of interaction has been reported with both related and unrelated viruses that infect a common host (51). Hamilton (22) has suggested that this type of interaction could result because the challenger replicates more slowly, or infects fewer cells in inducer-infected plants.

The results obtained with the five lines presumed to be resistant to CAMV-Mor and -Cyp suggested that either the cowpea lines are not homogeneous or there is pathogenic variability among isolates of CAMV. Due to the limited availability of seeds, only small numbers from each line were tested. Future study should include more seeds of single-plant selections from these lines.
from these lines.

A correct identification of the virus(es) infecting a crop is considered essential before adequate control measures can generally be developed. This would be especially critical with BlCMV and CAMV because different factors or genes confer resistance to each of these viruses. Although no common source of resistance to BlCMV and CAMV was identified in cowpea in this study, cowpea lines resistant to each of these viruses were identified. These sources of resistance can be used in breeding programs to develop resistant cultivars with desirable horticultural characteristics. Furthermore, these resistant lines can be used to separate these viruses when they occur in mixture, and they represent valuable diagnostic hosts for the correct identification of BlCMV and CAMV isolates.

The results obtained suggested that BlCMV is not confined to the United States as the literature suggested. The Kenya and Nigeria isolates of CAMV were identified as BlCMV isolates on the basis of their serological reactions (44) and host response. A seedborne virus of asparagus bean (*Vigna sesquipedalis* Fruw.) from Japan was also identified as a BlCMV isolate in serological tests (M. Taiwo, *unpublished*) and, recently, BlCMV was reported to occur in India (36). Using the same criterion, a virus isolated from cowpea by Kaiser et al (28) in Iran was confirmed to be CAMV (M. Taiwo, *unpublished*).

Aggregation of virus particles and virus and host components during purification have been identified as limiting factors in obtaining higher virus yields in the PVY group (3,25,43,47). The losses associated with low-speed centrifugation due to the aggregation of viral particles, was reduced by the addition of 0.01 M EDTA to the grinding buffer. EDTA reduces aggregation of papaya ringspot virus (PRV) in tissue extracts (20,50).

Capsid protein heterogeneity is a common phenomenon among potyviruses (25–27). The purification procedure and storage conditions of purified preparations influence the proteolytic conversion that causes this heterogeneity (25,26). A second centrifugation of BlCMV and CAMV in CsCl or Cs_2SO_4 before storage at 4 C seemed to prevent or minimize the proteolytic degradation of capsid protein.

Our results have clearly demonstrated that some viral isolates previously assumed to be CAMV were actually BlCMV. If effective control measures are to be developed, there is a need for the correct identification and determination of the actual geographical distribution of these viruses. If possible, the introduction of one or both viruses into areas where they do not exist now, must be prevented. The highly specific antisera produced to BlCMV and CAMV (44) and the resistant differential cowpea lines should help in such investigations.

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