

## Serological Grouping of Isolates of Blackeye Cowpea Mosaic and Cowpea Aphidborne Mosaic Viruses

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### ABSTRACT

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Antisera were prepared to two single-lesion isolates of blackeye cowpea mosaic virus (BICMV), one each from Florida and New York, and four single-lesion isolates of cowpea aphidborne mosaic virus (CAMV), one each from Cyprus, Kenya, Morocco, and Nigeria. Enzyme-linked immunosorbent assay (ELISA), immune electron microscopy (IEM), and immunodiffusion in sodium dodecyl sulfate (SDS) were used to compare these isolates. Results indicated two serological groups, which we defined as BICMV and CAMV. The BICMV group was comprised of both isolates of BICMV and the Kenya and Nigeria isolates of CAMV. The CAMV group contained the Morocco and Cyprus isolates. In all tests, isolates within a group showed very close, if not identical, serological relationship. Antisera to isolates of the CAMV group did not detectably react to isolates of the BICMV group. Similarly, antisera to the Florida and Nigeria isolates did

not react with members of our CAMV group in SDS-immunodiffusion and ELISA. However, antisera from bleedings taken 16 wk or more after injecting rabbits with the New York and Kenya isolates reacted weakly with Morocco and Cyprus isolates in SDS-immunodiffusion tests. Antisera to all isolates in our BICMV group moderately decorated the isolates in our CAMV group, but no decoration was observed in the reciprocal test. In reciprocal SDS-immunodiffusion tests, antisera to isolates in our BICMV group reacted strongly with bean common mosaic virus (BCMV) with slight spurs, whereas BCMV antiserum gave reactions of homology to all four antigens. No reaction to BCMV was detected in SDS-immunodiffusion tests when antisera to isolates in the CAMV group was used. BCMV antiserum, however, gave a weak reaction to CAMV isolates in reciprocal tests.

Blackeye cowpea mosaic virus (BICMV) and cowpea aphidborne mosaic virus (CAMV) are regarded as two distinct potyviruses (13,17). In nonreciprocal sodium dodecyl sulfate (SDS)-immunodiffusion tests conducted by using antiserum to BICMV, Lima et al (13) demonstrated that BICMV and CAMV are two distinct viruses. Serological relationships of isolates of both CAMV and BICMV to some potyviruses have also been reported (3,13,15,25). Some isolates of CAMV and BICMV are serologically related to bean common mosaic virus (BCMV), and BICMV is also related to bean yellow mosaic virus (BYMV), soybean mosaic virus (SMV), potato virus Y (PVY), tobacco etch virus (TEV), watermelon virus-2 (WMV-2), dasheen mosaic virus (DMV), and lettuce mosaic virus (LMV) (13). The CAMV isolates described by Bock (3) did not show any serological relationship to BYMV, SMV, PVY, and tobacco severe etch virus.

Our preliminary serological tests indicated BICMV and a strain of CAMV described by Bock (3) and Bock and Conti (4) were serologically identical. We therefore expanded our study and determined serological relationships between four isolates of CAMV, one each from Cyprus, Morocco, Kenya, and Nigeria, and two isolates of BICMV, one each from Florida and New York, to obtain more information for the proper classification of isolates of these two viruses.

Based on the results of reciprocal serological tests, we propose that the Kenya and Nigeria isolates previously designated as CAMV be considered isolates of BICMV, while the Morocco and Cyprus isolates be regarded as CAMV. This grouping also corresponds with the grouping of the six isolates by their differential host range on selected cowpea lines (23).

### MATERIALS AND METHODS

**Source and maintenance of virus isolates.** The BICMV isolates from Florida (D. Purcifull) and New York (J. K. Uyemoto) were designated BICMV-Fla2 and -Flo, respectively. The CAMV isolates 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), were designated as CAMV-Cyp, -Ken, -Mor, and -Nig, respectively. After three successive single-lesion transfers on *Chenopodium quinoa* Willd., the six isolates were maintained on California Blackeye peas.

**Purification and polyacrylamide gel electrophoresis of viral coat protein.** The six isolates were purified as described by Taiwo et al (23). Coat protein of purified virus preparations used in antiserum production was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Taiwo et al (23).

**Preparation and titration of antisera.** Antisera to all six isolates were prepared by intramuscular injection of New Zealand white rabbits with purified virus emulsified with Freund's incomplete adjuvant (1:1, v/v). The rabbits were injected twice during the first week with ~2 mg of freshly purified virus on each occasion. This was followed by two other 1-2 mg injections, one 3 wk and the other 8 wk after the first injection. Thereafter, booster shots were given when the antiserum titer decreased. Freshly purified preparations were usually used for booster injections; but with BICMV-Flo and CAMV-Ken, preparations stored at 4 C for 2 mo were used. Two or three purified preparations were used for each rabbit. Rabbits were bled every 7-14 days after the third injection, for a period of 4-6 mo. Antisera titers were determined in SDS-immunodiffusion tests (19). Antisera reacting with healthy cowpea plant extract were cross-absorbed as described by Purcifull et al (21).

**Serological comparison of isolates.** The serological relationship between BICMV and CAMV was established using ELISA, immune electron microscopy (IEM), and SDS-immunodiffusion

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tests. All serological tests were done at least twice for each serum with different batches of antigens generally used in each run. Immunodiffusion tests were performed in agar plates prepared according to Purcifull and Batchelor (19). The medium consisted of 0.8% ion agar, 1.0% NaN<sub>3</sub>, and 0.5% SDS. Wells in the agar plates were cut with an Autogel T/M punch (Grafar Corp., Detroit, MI 48238) with a pattern of six peripheral and one central well (7 mm in diameter). Peripheral and central wells were 5 mm from edge to edge. The antigens were at 250 µg/ml purified virus or infected tissue (1 g/2 ml) ground in 0.5% SDS.

ELISA was performed as outlined by Clark and Adams (5) except that 0.1 M potassium phosphate + 0.1 M ethylenediamine tetracetic acid (EDTA) adjusted to pH 7.5 was substituted for extraction buffer. Polystyrene microtiter plates (Dynatech Lab., Inc., Alexandria, VA 22314) were used for ELISA tests. The reaction of the substrate was stopped after 30 min by adding 50 µl of 3 M NaOH. Absorbance was measured in a Beckman Model 25 spectrophotometer at 405 nm. At least two wells were filled per sample in each plate. Controls included buffer only, extracts of healthy and known infected tissues, or purified virus.

Immune electron microscopy was performed as outlined by Milne and Luisoni (18). Antisera to all six isolates were diluted 1:10 or 1:50 with 0.1 M potassium phosphate buffer, pH 7. Grids were floated on the diluted antisera for 5 min, and rinsed with 20 drops of phosphate buffer. The grids were then floated on purified virus (10 µg/ml) for 15 min, rinsed with 20 drops of buffer, and floated on the diluted antisera for 15 min. They were then rinsed with 20 drops

of buffer and 30 drops of distilled water before staining with 2% phosphotungstic acid. The dried grids were examined with a JEOL JEM 100B electron microscope.

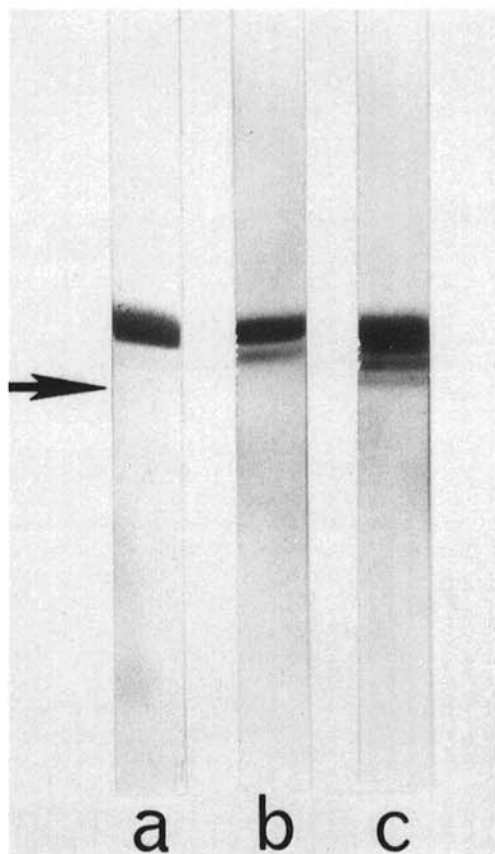
## RESULTS

**Characterization of antisera and proteins used for their preparation.** Capsid protein of potyviruses may be degraded by proteolysis during storage (7,20), and serological reactions of intact protein can differ from that of the degraded protein (7). Therefore, coat protein of purified virus used for immunizing rabbits was monitored by SDS-PAGE. Most of the preparations had one or two bands in SDS-PAGE (Fig. 1a and b), and the slowest migrating protein (Fig. 1a-c) predominated. One purified preparation of BICMV-Fla2 used for immunization, however, had three bands (Fig. 1c). Molecular weights estimated for these bands in 7.5% gels were 34,000-35,000, 31,000, and 29,000 for top, middle, and bottom bands, respectively (23).

Antisera prepared against CAMV and BICMV reacted with SDS-degraded purified virus preparations or sap from infected plants. Titers of the antisera varied from one rabbit to another and depended on the time between injection and bleeding. In general, reciprocals of antisera titers were four or eight after the first three injections, but increased to eight or 16 after a booster injection.

**Comparison of isolates in SDS-immunodiffusion tests.** The results of reciprocal comparison of all the isolates indicated that BICMV-Fla2, -Flo, CAMV-Ken, and -Nig were serologically identical in SDS-immunodiffusion tests (Fig. 2; Table 1). Similarly, CAMV-Mor and -Cyp reacted identically to homologous and heterologous antisera in SDS-immunodiffusion test (Fig. 2), but not identically to the other CAMV isolates or the two BICMV isolates.

Antisera from bleedings obtained 6-12 wk after the first injection with BICMV-Fla2, -Flo, CAMV-Ken, and -Nig did not react with CAMV-Mor or -Cyp (Figs. 2 and 3a). However, antisera from later bleedings (ie, 16 wk after the first injection) from rabbits immunized with BICMV-Flo and CAMV-Ken gave weak reactions with CAMV-Mor and -Cyp in SDS-immunodiffusion test (Fig. 3b). Reactions with definite spurs occurred when the same antisera were reacted with 2.5% pyrrolidine degraded antigens in a medium containing 0.75% ion agar, 0.85% NaCl, and 0.02 M NaN<sub>3</sub> (Fig. 3c). None of the bleedings from rabbits immunized with CAMV-Mor or -Cyp cross-reacted with BICMV-Fla2, -Flo, and CAMV-Ken



**Fig. 1.** Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of SDS-dissociated virus preparations used for production of antisera to blackeye cowpea (BICMV) and cowpea aphidborne mosaic viruses (CAMV). Gel a, Kenya isolate of CAMV; gel b, New York isolate of BICMV; gel c, Florida isolate of BICMV. Arrow marks the position of the marker protein carbonic anhydrase (MW 29,000). Electrophoresis was from top to bottom in a 7.5% gel. Capsid protein from CAMV from Morocco, Cyprus, and Nigeria showed bands similar to those represented by gel b. The molecular weights estimated were ~34,400 for the component in a; 34,900 and 30,500 for the components in b; and 34,900, 31,800, and 29,000 for the components in c.

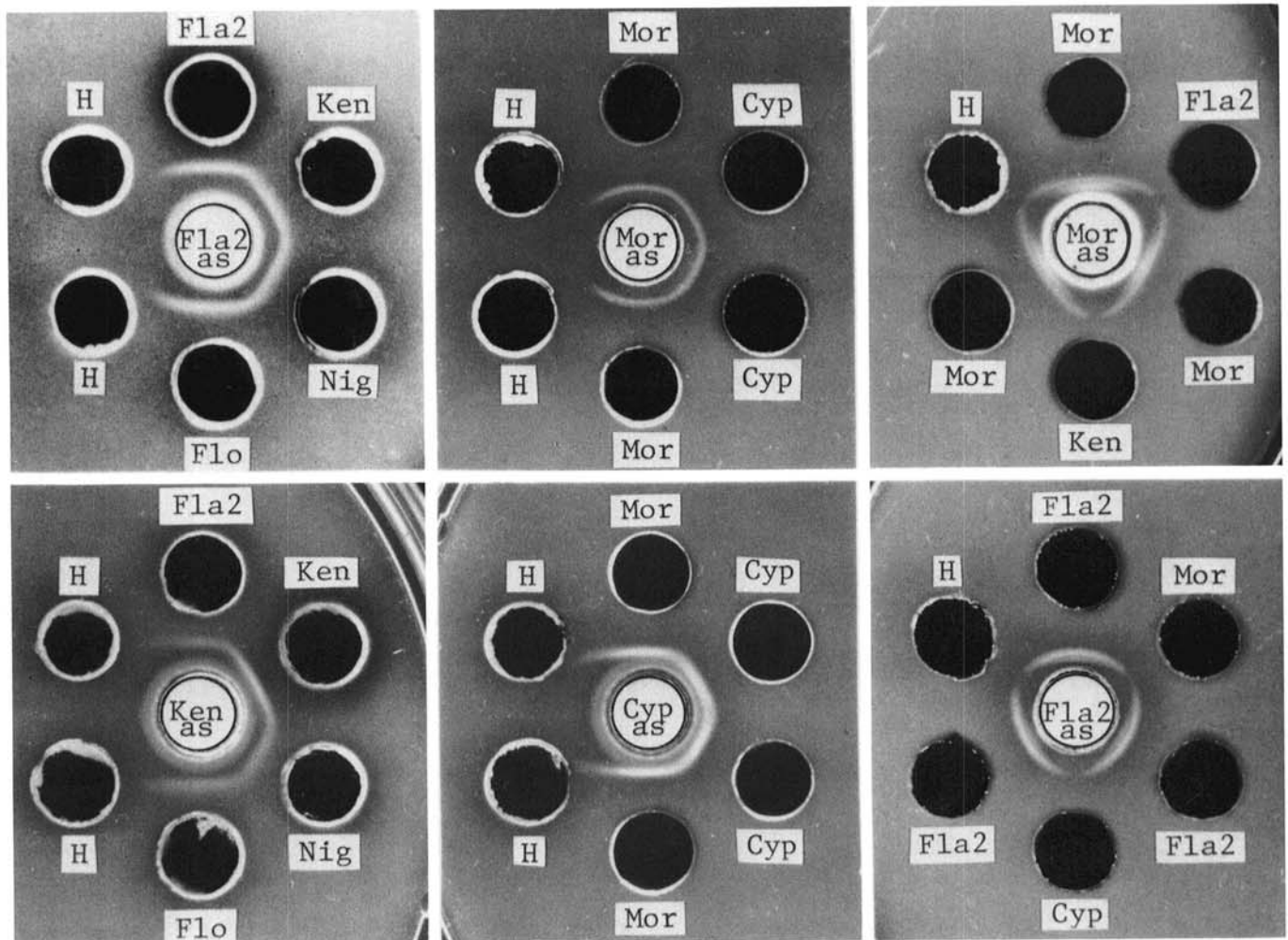
**TABLE 1.** Reaction of blackeye cowpea mosaic (BICMV) and cowpea aphidborne mosaic virus (CAMV) to homologous and heterologous antisera and to antisera of other potyviruses, in sodium dodecyl sulfate immunodiffusion tests

Antisera <sup>a</sup>	Antigens <sup>a</sup>									
	Fla2	Flo	Ken	Nig	Mor	Cyp	BCMVB	PVY	TEV	SMV
Fla2	+ <sup>b</sup>	+	+	+	-	-	+ <sup>c</sup>	-	-	-
Flo	+	+	+	+	+ <sup>c</sup>	+ <sup>c</sup>	+ <sup>c</sup>	-	-	-
Ken	+	+	+	+	+ <sup>c</sup>	+ <sup>c</sup>	+ <sup>c</sup>	-	-	-
Nig	+	+	+	+	-	-	+ <sup>c</sup>	-	-	-
Mor	-	-	-	-	+	+	-	-	-	-
Cyp	-	-	-	-	+	+	-	-	-	-
BCMVB	+	+	+	+	+ <sup>c</sup>	+ <sup>c</sup>	+	N	N	N
PVY	-	-	-	-	-	-	N	+	N	N
TEV	+ <sup>c</sup>	+ <sup>c</sup>	+ <sup>c</sup>	+ <sup>c</sup>	-	-	N	N	+	N
SMV	-	-	-	-	-	-	N	N	N	+
BICMV <sup>c</sup>	+	+	+	+	+ <sup>c</sup>	+ <sup>c</sup>	+ <sup>c</sup>	+ <sup>c</sup>	-	+ <sup>c</sup>

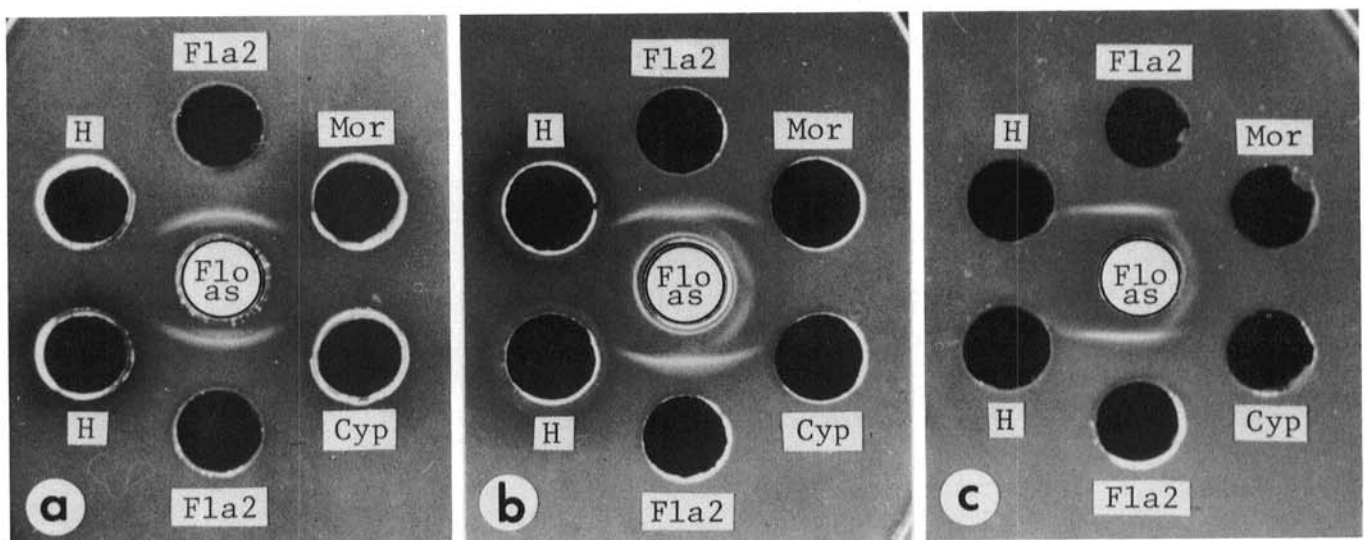
<sup>a</sup> Viral isolates and antisera used: Fla2 and Flo are the Florida and New York isolates of BICMV; Ken, Nig, Mor, and Cyp are the Kenya, Nigeria, Morocco, and Cyprus isolates of CAMV, respectively. BCMVB = bean common mosaic virus, PVY = potato virus Y, TEV = tobacco etch virus, and SMV = soybean mosaic virus.

<sup>b</sup> + = Reaction of homology or identity. - = No reaction. +<sup>c</sup> = Weak heterologous reaction with homologous reaction lines spurring over heterologous. +<sup>c</sup> = Strong reaction with homologous reaction lines spurring over heterologous antigen. N = Not tested.

<sup>c</sup> Antisera to BICMV produced by Lima et al (13).



**Fig. 2.** Sodium dodecyl sulfate (SDS)-immunodiffusion tests with two isolates of blackeye cowpea mosaic virus (BICMV) and four isolates of cowpea aphidborne mosaic virus (CAMV). Center and peripheral wells were filled with antisera, and virus (250  $\mu$ g/ml) or healthy plant sap, respectively. BICMV isolates were: Florida (Fla2) and New York (Flo). CAMV isolates were: Kenya (Ken), Morocco (Mor), Cyprus (Cyp), and Nigeria (Nig). Antisera of the isolates were designated with an additional "as." Note that the serological reactions indicate identity between Fla2, Flo, Ken, and Nig, and between Mor and Cyp. Reactions (not shown) of antisera to the other isolates confirmed the above grouping.



**Fig. 3.** Factors affecting the cross-reactivity of antisera made to the New York (Flo) isolate of blackeye cowpea mosaic virus (BICMV) with antigens of Morocco (Mor) and Cyprus (Cyp) isolates of cowpea aphidborne mosaic virus (CAMV). Tests **a** and **b** were in sodium dodecyl sulfate immunodiffusion plates. Test **c** was in a plate containing 0.75% ion agar, 0.85% NaCl and 0.02 M  $\text{NaN}_3$ . Purified virus (250  $\mu$ m/ml) and healthy cowpea leaf extracts (H) were used in the peripheral wells of **a** and **b**. The same was used in **c** except that the antigen preparations contained 2.5% pyrrolidine. Antiserum in test **a** was from a bleeding taken 2 mo after the first injection, while **b** and **c** contained antiserum to a 6-mo bleeding. Note that there were no heterologous reactions in **a**, whereas there were in **b** and **c**. Similar cross-reactions were observed with antiserum made to CAMV-Kenya. See Fig. 1 for serological reactions of BICMV and CAMV isolates in SDS tests.

and -Nig.

Antisera to both BICMV isolates, CAMV-Ken, and -Nig reacted strongly with BCMV, with homologous reaction lines spurring slightly over heterologous lines (representative reactions are shown in Fig. 4). Antiserum to BCMV gave reactions of homology between BCMV antigens and antigens of all four isolates (Fig. 4). Antiserum to BCMV reacted very weakly to CAMV-Mor and -Cyp, but reciprocal tests were negative.

The results of reciprocal serological tests with other potyviruses indicated that both isolates of BICMV, CAMV-Ken, and -Nig were not serologically related to PVY and SMV (Table 1). Unilateral serological reactions (ie, homologous lines spurred over heterologous) were detected with these isolates and TEV antiserum. CAMV-Mor and -Cyp were not related to PVY, TEV, and SMV. The antiserum produced to intact BICMV by Lima et al (13) reacted with CAMV-Mor, -Cyp, BCMV, PVY, and SMV with homologous reaction lines spurring over heterologous lines (Table 1; Fig. 5).

**Comparison of isolates by ELISA.** Preliminary ELISA tests with BICMV-Fla2 or CAMV-Mor and their homologous antiserum gave low  $A_{405}$  values. Since the sensitivity of ELISA in detection of papaya ringspot virus is affected by the extraction buffer used (16), we tested effects of different extraction buffers for BICMV and CAMV. Addition of 0.1 M EDTA to the standard ELISA buffer improved sensitivity of ELISA by about fourfold for both viruses (Table 2). Potassium phosphate enhanced the sensitivity even more. Therefore, subsequent ELISA tests were performed using 0.1 M potassium phosphate + 0.1 M EDTA, pH 7.5, as the extraction or dilution buffer.

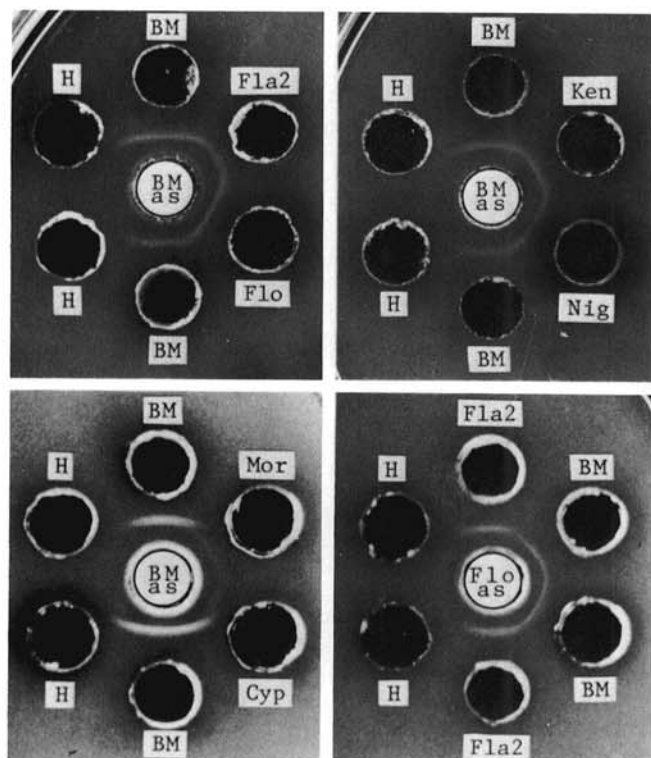
ELISA was performed with antisera prepared to BICMV-Fla2 and to CAMV-Mor. The two isolates of BICMV, CAMV-Ken, and -Nig reacted strongly to BICMV-Fla2 antiserum irrespective of

$\gamma$ -globulin (Fig. 6.) and antigen concentrations (*unpublished*). Similarly, the Mor and Cyp isolates of CAMV reacted strongly to CAMV-Mor antiserum (Fig. 6). No cross-reactions between the two groups were detected. Cross-reaction was not detected even when CAMV-Ken antiserum from a 16-wk bleeding from the injected rabbit was used in ELISA (*unpublished*). This same bleeding gave a weak reaction to CAMV-Mor and -Cyp in SDS-immunodiffusion tests (Fig. 3b).

**Comparison of isolates using IEM.** Antisera to BICMV-Fla2, -Flo, CAMV-Ken, and -Nig decorated all four antigens heavily (Fig. 7a-c). Interestingly, CAMV-Mor and -Cyp were moderately decorated by these antisera (Fig. 7d). However, no decoration was observed when a serologically unrelated virus like papaya ringspot virus was reacted with these antisera (Fig. 7e). CAMV-Mor reacted identically to homologous and CAMV-Cyp antisera, and vice versa (Fig. 7f to h). However, CAMV-Mor and -Cyp never reacted with BICMV-Fla2, -Flo, CAMV-Ken, and -Nig (Fig. 7i).

## DISCUSSION

This study has demonstrated that the Kenya and Nigeria isolates of CAMV are serologically identical to BICMV. The results of the biological characterization of these two isolates have also shown that they are indistinguishable from the two BICMV isolates (23). Since BICMV-Fla2, -Flo, CAMV-Ken, and -Nig are serologically identical and share a common host range, we suggest that the Kenya and Nigeria isolates of CAMV be regarded as BICMV isolates. The results of this study also indicate that CAMV-Mor and -Cyp are serologically identical and are distantly related to BICMV. These two isolates also have a fairly similar host range, and we have proposed that they be retained as CAMV isolates (23). With the availability of antisera to both BICMV and CAMV, we believe more CAMV isolates will be identified as BICMV and vice



**Fig. 4.** Serological reactions of bean common mosaic virus (BCMV) antiserum with two isolates of blackeye cowpea mosaic virus (BICMV) and four isolates of cowpea aphidborne mosaic virus (CAMV) in sodium dodecyl sulfate (SDS)-immunodiffusion tests. Center wells were filled with BCMV antiserum (BM as) and an antiserum prepared against the New York isolate of BICMV (Flo as). Peripheral wells were filled with BCMV (BM) from infected beans, prepared in 1.0% SDS; 250  $\mu$ g/ml of purified BICMV isolates from Florida (Fla2) and New York (Flo), CAMV isolates from Kenya (Ken), Nigeria (Nig), Morocco (Mor), and Cyprus (Cyp); or healthy sap (H).

**TABLE 2.** Effect of extraction buffers on the sensitivity of enzyme-linked immunosorbent assay (ELISA) for detecting blackeye cowpea mosaic (BICMV) and cowpea aphidborne mosaic viruses (CAMV) with homologous antisera<sup>a</sup>

Buffers	Absorbance <sup>b</sup> at 405 nm obtained by using tissue that was:			
	BICMV-infected <sup>c</sup>	Healthy	CAMV-infected <sup>d</sup>	Healthy
0.4 M PB <sup>e</sup>	1.59	0.10	0.63	0.01
0.4 M PB + 0.1 M EDTA <sup>f</sup>	2.35	0.09	0.48	0.02
0.25 M PB	2.26	0.09	0.53	0.01
0.25 M PB + 0.1 M EDTA	2.14	0.10	0.66	0-0.07
0.1 M PB	1.43	0.12	0.32	0.06
0.1 M PB + 0.1 M EDTA	2.02	0.10	0.77	0.03
0.1 M EDTA	0.69	0.07	0.73	0.06
0.01 M EDTA	0.09	0.07	0.14	0.04
0.001 M EDTA	0.05	0.04	0.09	0.05
Std EB <sup>g</sup>	0.38	0.11	0.21	0.03
Std EB + 0.1 M EDTA	1.76	0.07	0.83	0.01
Std EB + 0.01 M EDTA	0.47	0.07	0.14	0.03
Std EB + 0.001 M EDTA	0.57	0.08	0.16	0.04
Water	0.05	0.03	0.18	0.03

<sup>a</sup>ELISA was performed with coating  $\gamma$ -globulin at 5  $\mu$ g/ml; healthy and infected tissues were homogenized in the different buffers at a ratio of 1:20. Enzyme-conjugated  $\gamma$ -globulin was at 1/400 dilution; the substrate *p*-nitrophenyl phosphate was at 0.6 mg/ml, the content of each well was diluted to twice its volume before the absorbance was determined spectrophotometrically. Antiserum to BICMV and to CAMV was used. Only homologous combinations were tested.

<sup>b</sup>Absorbance values are averages from two wells. These values are from one experiment. Other experiments gave similar results.

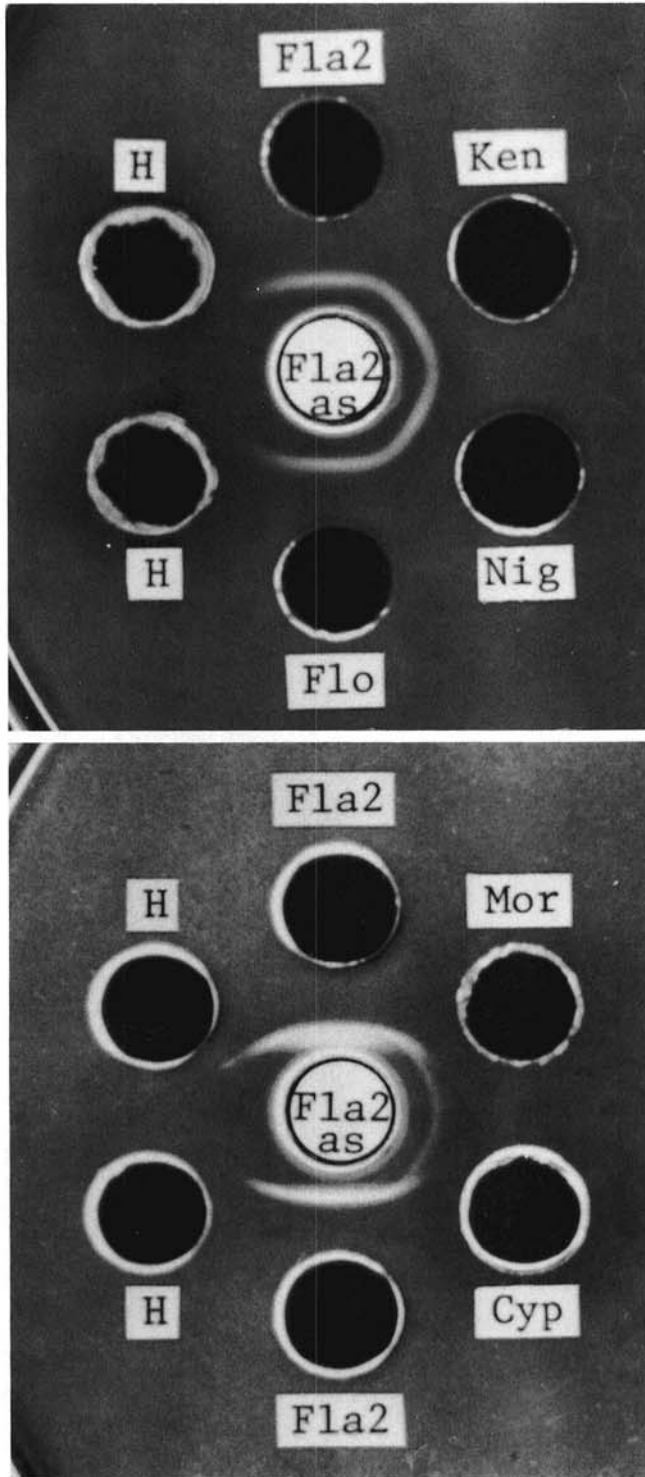
<sup>c</sup>Florida isolate of BICMV.

<sup>d</sup>Morocco isolate of CAMV.

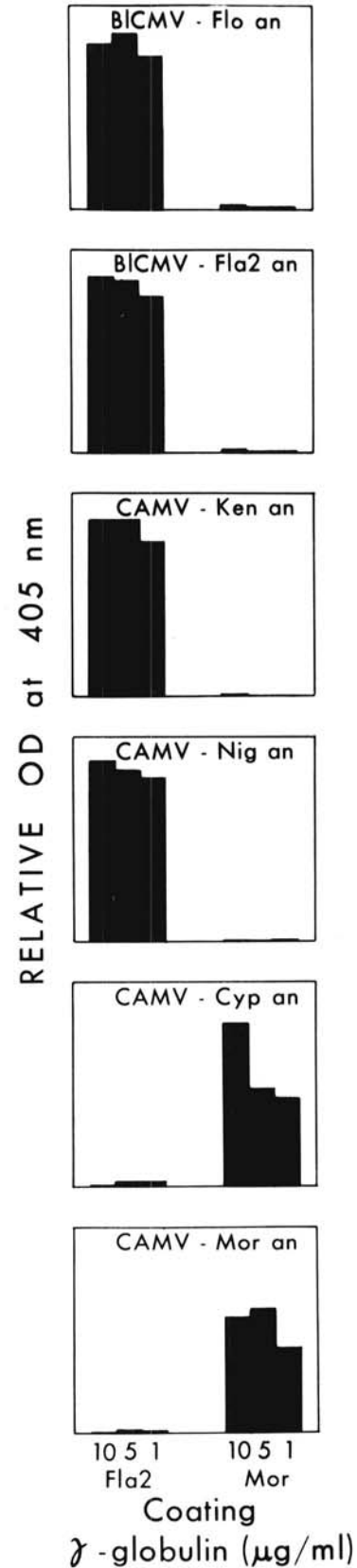
<sup>e</sup>PB = potassium phosphate, pH 7.5.

<sup>f</sup>EDTA = ethylenediamine tetracetic acid, disodium salt.

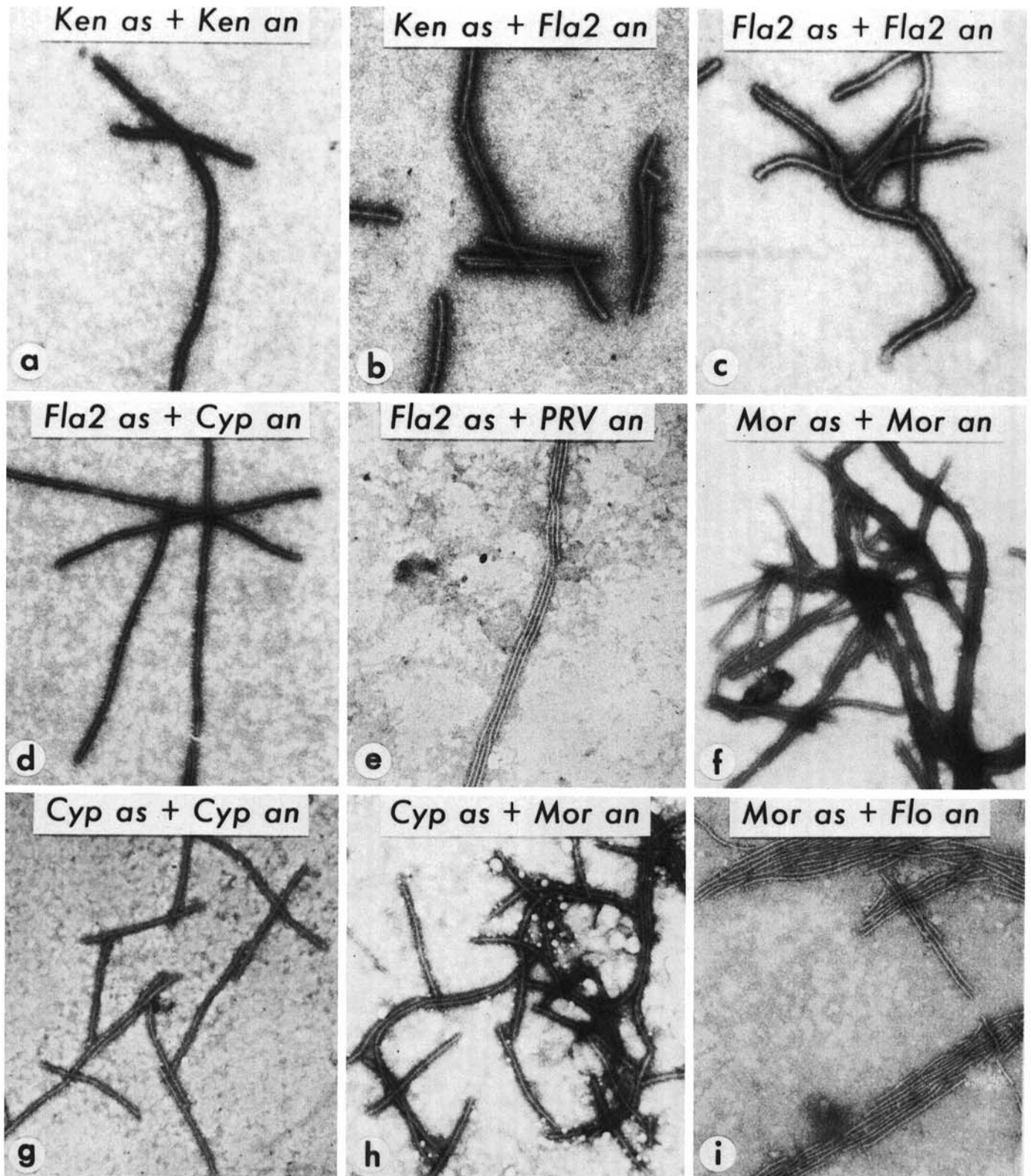
<sup>g</sup>Std EB = Standard ELISA buffer (0.02 M potassium phosphate + 0.15 M NaCl + polyvinyl pyrrolidone + 0.005% Tween-20 + 0.02% Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>), pH 7.4.



**Fig. 5.** The serological reaction of two isolates of blackeye cowpea mosaic virus (BICMV) and four isolates of cowpea aphidborne mosaic virus (CAMV) to antiserum produced to BICMV by Lima et al (13) in sodium dodecyl sulfate-immunodiffusion tests. Center and peripheral wells were filled with suspensions of antiserum and virus (250  $\mu\text{g}/\text{ml}$ ) or healthy sap (H), respectively. BICMV isolates were: Florida (Fla2) and New York (Flo). CAMV isolates were: Kenya (Ken), Morocco (Mor), Cyprus (Cyp), and Nigeria (Nig). Note that the serological reactions indicate identity between Fla2, Flo, Ken, and Nig, thus confirming results presented in Fig. 2; also note the strong reaction between their antiserum and CAMV-Mor and -Cyp, in contrast to a lack of reaction between the antiserum we prepared to BICMV-Fla2 and CAMV-Mor and -Cyp (Fig. 2).



**Fig. 6.** Reaction of isolates of blackeye cowpea mosaic virus (BICMV) and cowpea aphidborne mosaic virus (CAMV) in enzyme-linked immunosorbent assay (ELISA) measured by using antisera to BICMV-Florida (Fla2) and CAMV-Morocco (Mor). Coating  $\gamma$ -globulin was at 10, 5, and 1  $\mu\text{g}/\text{ml}$ ; enzyme conjugate was at 1/400 dilution; and purified virus was at 5  $\mu\text{g}/\text{ml}$ . Antigens were: BICMV-Florida (Fla2) and New York (Flo); and CAMV-Morocco (Mor), Nigeria (Nig), Kenya (Ken), and Cyprus (Cyp). Prior to graphing, healthy background values were subtracted from the virus reaction values.



**Fig. 7.** Reaction of isolates of blackeye cowpea mosaic virus (BICMV) and cowpea aphidborne mosaic virus (CAMV) to homologous and heterologous antisera in immune electron microscopy. Electron micrographs of grids prepared with **a**, antiserum to Kenya isolate of CAMV (Ken as) and homologous antigen (Ken an); **b**, antiserum to CAMV-Ken (Ken as) and Florida isolate of BICMV (Fla2 an); **c**, antiserum to BICMV-Fla2 (Fla2 as) and homologous antigen (Fla2 an); **d**, antiserum to BICMV-Fla2 (Fla2 as) and the Cyprus isolate of CAMV (Cyp an); **e**, antiserum to BICMV-Fla2 (Fla2 as) and unrelated papaya ringspot virus (PRV an); **f**, antiserum to Morocco isolate of CAMV (Mor as) and homologous antigen (Mor an); **g**, antiserum to CAMV-Cyp (Cyp as) and homologous antigen (Cyp an); **h**, antiserum to CAMV-Cyp (Cyp as) and CAMV-Mor (Mor an); and **i**, antiserum to CAMV-Mor (Mor as) and New York isolate of BICMV (Flo an). Purified virus was used as antigen in every case. Magnifications: **a-h**,  $\times 35,000$ ; **i**,  $\times 27,300$ .

versa.

Our results of reciprocal SDS-immunodiffusion tests with antisera to BICMV-Fla2, -Flo, CAMV-Ken, -Nig, -Mor, -Cyp, and BCMV confirmed the results of previous work showing that BICMV is very closely related to BCMV (25) and that a closer serological relationship exists between BICMV and BCMV than between BICMV and CAMV (13).

Failure of the six antisera to react with such potyviruses as TEV, PVY, and SMV agree with findings of Bock (3), but disagree with the results of Lima et al (13). Our results differ from those of Lima et al (13) in these respects: stronger heterologous reactions were observed between their antiserum to BICMV and CAMV-Mor; and their antiserum to BICMV cross-reacted with several other potyviruses including TEV, PVY, SMV, BCMV, BYMV, WMV-2, DMV, and LMV.

There may be a number of reasons for these discrepancies. Variations in the degree of cross-reactivity exhibited by antisera obtained from rabbits injected with the same amount of immunogen have been observed by several workers (10,22,24,27). This has been attributed to differences between individual animals (27), the route and number of injections given in the immunization program (8), and the time of bleeding after the first immunization (11,24,27). Any of these and perhaps other factors may have contributed to the differences in our results.

The extraction buffer used in the ELISA test affected its sensitivity. Presence of EDTA in the buffer has been shown to prevent or reduce the aggregation of papaya ringspot virus (6,28). It probably plays a similar role in this system.

In ELISA tests, reactions were not observed between antisera to BICMV-Fla2, -Flo, CAMV-Ken, and -Nig; and antigens of CAMV-Mor and -Cyp; and vice versa. Failure to detect serologically related viruses in ELISA with the double antibody sandwich method has been reported by others (1,2,9,14,26). The indirect ELISA technique has been used to detect a wide range of TMV strains with antiserum to only one strain (26). This technique would be useful for field detection of BICMV and CAMV isolates.

Our results suggest that IEM may be more sensitive in detecting low levels of cross-reacting antibodies than SDS-immunodiffusion tests or ELISA.

The results of this investigation demonstrated that the Kenya and Nigeria potyviruses described by Bock (3) and Ladipo (12), respectively, are serologically identical to BICMV, which until very recently (16) has only been reported in the United States. Our results also suggest that the Morocco and Cyprus isolates of CAMV are serologically identical and are very distantly related to BICMV. Implications of these results on geographical distribution, classification, and breeding for resistance to BICMV and CAMV are discussed in a concurrent paper (23).

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