Purification, Serology, and Some Physical Properties of Dasheen Mosaic Virus

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This research was supported in part by U.S. Department of Agriculture Cooperative Agreement No. 12-14-7001-284 and in part by BMS Grant No. 75-14014 from the National Science Foundation. Appreciation is expressed to the Pan American Plant Co., Products Division of Geo. J. Ball, Inc., West Chicago, IL 60185, for supplying *Philodendron selloum* seedlings. Potato Y, tobacco etch, turnip mosaic, and blackeye cowpea mosaic viruses and their antisera were provided by D. E. Purcifull and A. Lima, Department of Plant Pathology, University of Florida, Gainesville.

Journal Series Paper No. 385 of the Florida Agricultural Experiment Station.

Accepted for publication 15 June 1977.

ABSTRACT

ABO EL-NIL, M. M., F. W. ZETTLER, and E. HIEBERT. 1977. Purification, serology, and some physical properties of dasheen mosaic virus. Phytopathology 67:1445-1450.

A technique for purifying dasheen mosaic virus (DMV) is described. Antisera prepared to two DMV isolates were virus specific in immunodiffusion tests, and homologous titers of 1:512 were recorded for both isolates in microprecipitin tests. Dasheen mosaic virus is related serologically to blackeye cowpea mosaic and tobacco etch viruses, but not to potato Y or turnip mosaic viruses. The average uncorrected $A_{260/247}$ and $A_{260/280}$ ratio of each DM isolate was 1.08 ± 0.2 and 1.19 ± 0.2, respectively, and the A_{260} (1 mg/ml, 1-cm light path) was 2.38 ± 0.05. Polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate revealed coat protein heterogeneity and anomalous electrophoretic behavior. Coat protein molecular weights ranged from 25,000-45,000 in 11% polyacrylamide, 28,000-51,000 in 6% polyacrylamide, and 30,000-80,000 in 3% polyacrylamide.

Additional key words: Araceae, aroids, *Colocasia* spp., *Philodendron selloum*, potyvirus, techniques.

Dasheen mosaic virus (DMV) initially was described in 1970 as a potyvirus infecting members of the Araceae (23). Since then, it has been detected in commercial plantings of aroids in Florida (11), Egypt (1), Puerto Rico (4), Venezuela (6), Japan (21), The Netherlands (15), and the Solomon Islands (7, 17).

Dasheen mosaic virus has a mean particle length of approximately 750 nm, is transmitted by aphids in a stylet-borne manner, induces cytoplasmic inclusions resembling those of other potyviruses, and is serologically related to tobacco etch virus (9, 23). In *Philodendron selloum* leaf extracts, DMV was shown to have a dilution end point of 10^{-2} to 10^{-3}, a thermal inactivation point between 60 and 65 C, and longevity in vitro of 75 hr (3).

The current study describes (i) a method for purifying DMV from infected *Philodendron selloum* leaves and (ii) certain serological and physical properties of this virus.

MATERIALS AND METHODS

Three isolates of DMV were studied: (i) DMV-FL, originally from a subaquatic, noncultivated variety of *Colocasia esculenta* (L.) Schott growing in Gainesville, Florida; (ii) DMV-FJ, from edible taro (*C. esculenta* 'MuMu') propagated in the Fiji Islands; and (iii) DMV-E from Egyptian taro (*Colocasia antiquorum* Schott 'Shanawani') grown in Egypt. All three isolates were maintained in *Philodendron selloum* C. Koch seedlings growing in a greenhouse at Gainesville.

Plants from which DMV-FL and DMV-E were originally recovered had foliar mosaic symptoms like those described previously (1, 23). Symptoms of plants from which DMV-FJ was recovered, however, were much more pronounced (Fig. 1), and these plants always produced leaves with symptoms. Nevertheless, all three DMV isolates induced similar symptoms in aroid indicator plants (*Caladium hortulanum* Birdsey, *C. esculenta*, *Dieffenbachia picta* Schott, and *Xanthosoma caracu* Koch & Bouche) except that DMV-FJ induced a greater degree of stunting in *P. selloum* than the other two isolates (Abo El-Nil et al., *unpublished*). In addition, all three isolates were stylet transmissible by aphids [*Aphis craccivora* Koch and *Myzus persicae* (Sulzer)] (18), and have mean particle lengths approximately 750 nm (1, and Abo El-Nil et al., *unpublished*).

Young seedlings of *P. selloum* were used exclusively throughout this investigation. All plants were manually inoculated with extracts of infected leaves triturated in 0.02 M Tris-HCl [tris (hydroxy methyl) amino methane] buffer at pH 7.2. Carborundum (0.22 μm average particle size) was dusted on leaves of test plants or mixed with inoculum.

The viruses (DMV-FL and DMV-FJ) were purified from systemically infected *P. selloum* leaves approximately 2 wk after inoculation. One-hundred g of tissue were homogenized with a precooled Waring Blendor in a chilled mixture of 200 ml 0.1 M sodium citrate (pH 7.2)

containing 0.6 g sodium sulfite and 0.01 M Na_2EDTA (disodium ethylenediaminetetraacetate), 45 ml chloroform, and 45 ml carbon tetrachloride. The homogenate then was centrifuged at 13,200 *g* for 10 min, and the aqueous supernatant liquid was stirred for 4 hr at 4 C with 8% (w/v) polyethylene glycol 6000. The precipitated virus was pelleted by centrifugation at 13,200 g for 10 min, and the pellet was resuspended by stirring 15 hr at 4 C in 0.02 M Tris-HCl buffer (pH 7.2) containing 0.1% 2-mercaptoethanol (2-ME) and 0.01 M Na_2EDTA. The suspension then was centrifuged at 18,800 *g* for 10 min and the clear supernatant liquid was retained for equilibrium density-gradient centrifugation in cesium chloride (CsCl). One-half ml of virus suspension was layered upon 4.5 ml CsCl (mean density = 1.28 g/cm^3) in 0.02 M Tris buffer (pH 7.2) contained in 13 × 50-mm centrifuge tubes. The tubes were centrifuged at 105,800 *g* for 17 hr. After centrifugation, the opalescent zone 10-12 mm from the bottom of the gradient was extracted by piercing the wall of the centrifuge tube with a needle. The extracted material was resuspended in 0.02 M Tris-HCl buffer (pH 7.2) and 0.1% 2-ME and pelleted by centrifugation at 84,500 *g* for 2 hr. The pellet was resuspended in 0.02 M Tris-HCl buffer (pH 7.2) and further clarified by centrifugation at 6,600 *g* for 10 min.

Ultraviolet absorption spectra of the purified virus suspensions were determined using a Beckman Model 25 spectrophotometer. Ultraviolet absorption values at different wavelengths were determined for 10 preparations of each virus isolate (DMV-FL and DMV-FJ). The extinction coefficient of these two isolates was determined by the dry weight measurements (after oven-drying at 90 C) of two 0.02-ml samples of each virus suspended in water at a concentration of A_{260}=20.

The molecular weights (MW) of DMV-FL and DMV-FJ viral coat protein were estimated by the polyacrylamide gel electrophoresis technique as described by Hiebert and McDonald (13). Preparations of purified virus were dissociated for electrophoresis in sodium dodecyl sulfate (SDS) and compared with the following markers: (i) bovine serum albumin (MW 67,000), (ii) glutamate dehydrogenase (MW 53,000), (iii) ovalbumin (MW 43,000 (iv) carbonic anhydrase (MW 29,000), and (v) tobacco mosaic viral coat protein subunits (MW 17,500).

Virus suspensions were prepared routinely for examination with a Philips Model 200 electron microscope by placing them on Formvar-coated, carbon-stabilized grids and staining them with 1 or 2% potassium phosphotungstate at pH 7.7. Size determinations were made by comparing projected micrographs to a 2,160-line/mm diffraction grating.

Antisera to DMV-FL and DMV-FJ were prepared by injecting rabbits intramuscularly each week for 7 wk with 1 ml of purified virus suspension ($A_{260/280}$ of 1.18 – 1.2, A_{260}=5) emulsified with 1 ml of Freund's incomplete adjuvant. Serological relationships were determined by Ouchterlony agar-gel double-diffusion tests (5). When purified virus preparations were tested, the immunodiffusion medium contained 0.8% Ionagar, 0.5% SDS, and 1% sodium azide (8). When viral D-protein was tested, SDS was omitted from the medium; D-proteins of DMV-FL and DMV-FJ were prepared by degrading purified virus suspensions in 2.5% pyrrolidine (19).

Antigen wells, 6 mm in diameter, were located 5 mm from the antiserum wells. After the reactants were added, the plates were incubated at approximately 24 C in a humid chamber. Reactants were observed after 24 hr and photographed after 48 hr.

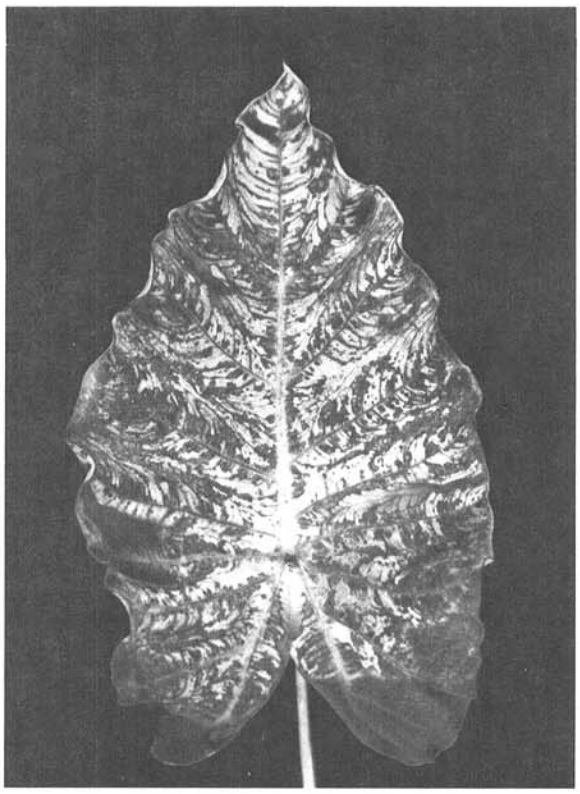

Fig. 1. Typical mosaic and distortion symptoms caused by dasheen mosaic virus (DMV) in taro cultivar MuMu from which the DMV-FJ isolate was originally recovered.

RESULTS

Purified preparations of DMV-FL and DMV-FJ were infectious at approximately A_{260}= 2 × 10^{-7} and particle fragmentation was evident (Fig. 2, Table 1). The average yields of seven trials were 6 and 20 mg of purified virus per 100 g of infected tissue for DMV-FL and DMV-FJ, respectively (calculated on the basis of an extinction coefficient at 260 nm of 2.38 and not corrected for light scattering).

The absorption spectra of purified preparations of each virus (suspended in 0.02 M Tris-HCl buffer at pH 7.2) revealed curves with the minima at 247 nm, maxima at 260 nm, and slight tryptophan shoulders at 290 nm characteristic of other filamentous viruses (Fig. 3). The average uncorrected $A_{260/247}$ and $A_{260/280}$ ratios were 1.08 ± 0.2 and 1.19 ± 0.2, respectively. The extinction coefficient of each virus at 260 nm was 2.38 ± 0.05.

The SDS-degraded coat proteins of DMV-FL and DMV-FJ, as analyzed by polyacrylamide gel electrophoresis (PAGE), revealed capsid protein heterogeneity (Fig. 4, 5; Table 2). Electrophoresis in 11% polyacrylamide gel resolved at least six electrophoretic components for

DMV-FJ with estimated molecular weights (MW) ranging from 45 × 10^3 to 25 × 10^3 and at least four electrophoretic components for DMV-FL ranging from 45 × 10^3 to 29 × 10^3. The two virus isolates differ in their electrophoretic patterns as indicated by the MW estimate of their second largest component (43 × 10^3 for DMV-FJ versus 39 × 10^3 for DMV-FL) and by the presence of a distinct component for DMV-FJ at 25 × 10^3. Electrophoresis also was done at gel concentrations of 3 and 6% to test for possible anomalous electrophoretic behavior of

TABLE 1. Length of dasheen mosaic virus (DMV-FJ) particles in leaf extracts or after purification using a chloroform/carbon tetrachloride mixture as clarifying agents[a]

Particle length (nm)	Distribution of total particles measured in: Leaf extracts (%)	Purified preparations (%)
106	0	6
132-211	0	26
238-290	0	12
317-356	0	20
422-502	0	11
528-607	0	9
634-713	10	5
739-792	63	8
818-898	26	1
924-1,003	1	0
1,030-1,109	0	0
1,135	0	2

[a]The values given represent 80 and 242 particles for leaf extracts and purified preparations, respectively. Sodium citrate (0.1 M) was used with the organic solvents. All virus particles were negatively stained in 1% potassium phosphotungstate prior to examination.

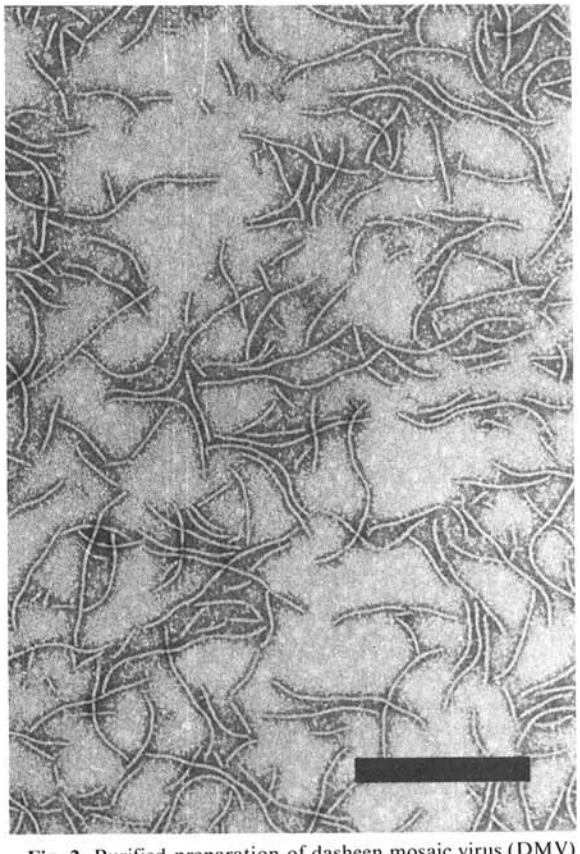

Fig. 2. Purified preparation of dasheen mosaic virus (DMV) isolate DMV-FJ stained in 1% potassium phosphotungstate. Scale line is 0.5 μm.

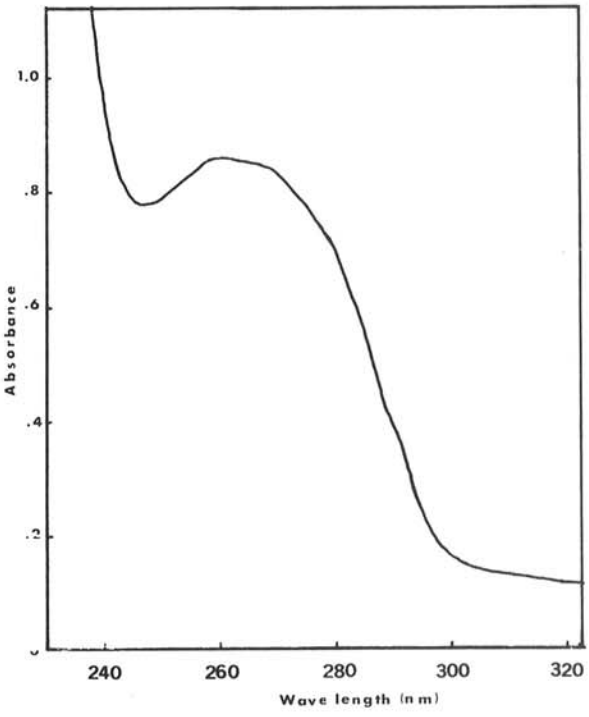

Fig. 3. Ultraviolet absorption spectrum of a purified preparation of dasheen mosaic virus (DMV) isolate DMV-FJ.

TABLE 2. Molecular weight (MW) estimates of DMV-FL and DMV-FJ coat proteins by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate

Isolate	Average MW estimates[a] in gel concentrations of: 11% (× 10^3d)	6% (× 10^3d)	3% (× 10^3d)
DMV-FL	45	51	76
	39	43	59
	37	28	30
	29	...	...
DMV-FJ	45	51	80
	43	46	65
	40	28	30
	37	...	...
	29	...	...
	25	...	...

[a]All values = × 10^3 daltons (d); molecular weight estimates are the averages for two separate experiments for the 11, 6, and 3% gels.

SDS-degraded capsid proteins. The results are summarized in Table 2 and in Fig. 4 and 5. Molecular weight estimates based upon movement in 3% acrylamide gels ranged from 80×10^3 to 30×10^3 for DMV-FJ and from 76×10^3 to 30×10^3 for DMV-FL; in 6% gels, MW estimates ranged from 51×10^3 to 28×10^3 for both isolates. These results clearly show that the capsid protein species larger than 30,000 behave anomalously during SDS-PAGE.

Antisera prepared to purified DMV-FL and DMV-FJ had titers of 1:512 against purified virus at $A_{260} = 0.5$ when tested by the twofold serial dilution microprecipitin method (5). The Ouchterloy agar double-diffusion method (5) was used to test the serological relationships between the DMV isolates and four other potyviruses. Isolates DMV-FL and DMV-FJ were serologically related when tested with their respective antisera in immunodiffusion plates containing 0.5% SDS in the medium (Fig. 6). Precipitin reactions were noted either when purified antigen ($A_{260} = 0.5$) or infective leaf extracts of *P. selloum*, dasheen, Egyptian taro, or malanga were tested. No reactions were noted, however, between antigen wells and wells containing normal serum.

Antiserum of DMV-FL reacted with its homologous antigen by forming a spur over the heterologous DMV-FJ antigen. This spur was noted regardless of whether SDS was incorporated in the medium or was omitted and the antigen degraded with pyrrolidine (Fig. 6).

Leaf extracts of *P. selloum* and Egyptian taro infected with DMV-E reacted positively to either DMV-FL or DMV-FJ antisera. A spur was noted when DMV-E antigen was tested against antiserum to DMV-FL, but not with DMV-FJ.

Neither antigens nor antisera of potato Y or turnip mosaic viruses reacted with any of the DMV antigens or antisera, although each of these viruses reacted with their respective homologous antisera. Precipitin reactions were noted, however, between antigens and antisera of DMV and antigens and antisera of tobacco etch (data not shown) or blackeye cowpea mosaic viruses (Fig. 6).

DISCUSSION

This study is the first report describing a technique for purifying DMV; and by using purified preparations, it was possible to determine certain physical properties of this virus for the first time (i.e., an $A_{260/280}$ ratio of 1.19 and extinction coefficient at 260 nm of 2.38). Antisera prepared to purified DMV proved to be highly reactive which enabled us to determine serological relationships of this virus to two other potyviruses (blackeye cowpea mosaic and tobacco etch). All these properties conform to properties described for other potyviruses, such as tobacco etch (16, 20), and support the original assertion that DMV is a member of the potato Y virus group (23).

Capsid protein heterogeneity as revealed by SDS-PAGE of the DMV isolates has been found with capsid proteins of other PVY group viruses (13, 14, 16). However, the MW estimates of two of the capsid protein species of the DMV isolates are considerably larger than

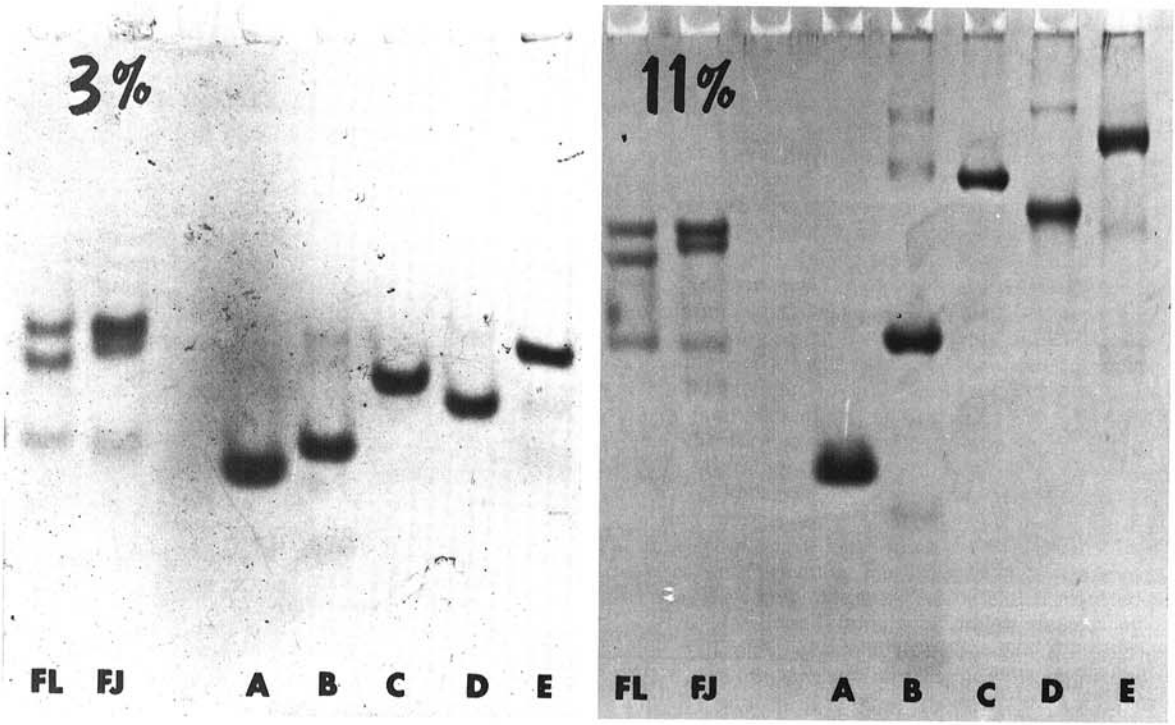

Fig. 4. Electrophoresis of dasheen mosaic virus (DMV) isolates DMV-FL (FL) and DMV-FJ (FJ) in 3 and 11% polyacrylamide gel slabs. Both gels contained 0.1% sodium dodecyl sulfate and sodium phosphate buffer (pH 7.2). Marker proteins are: (A) tobacco mosaic viral coat protein subunits (MW 17,500), (B) carbonic anhydrase (MW 29,000), (C) glutamate dehydrogenase (MW 53,000), (D) ovalbumin (MW 43,000), and (E) bovine serum albumin (MW 67,000). Electrophoresis is from top to bottom.

the values obtained for other PVY group viruses (43-45 × 10^3 for DMV versus 33-36 × 10^3 in 11% polyacrylamide gels) (14). In view of the obvious anomalous SDS-PAGE behavior of the larger components of the capsid protein of the DMV isolates and because of the lack of any apparent difference in sedimentation rate (Abo El-Nil et al., *unpublished*), UV spectrum, particle dimensions, etc., compared to other PVY group viruses, it is very likely that the capsid protein subunit size of DMV is similar (33-36 × 10^3) to that of other PVY group viruses.

The capsid proteins of a number of PVY group viruses examined to date have a similar SDS-PAGE mobility over a range of gel concentrations and have a similar MW estimate of about 27-29 × 10^3 after the labile protein portion is lost [this report and (13)]. It appears that the labile portion of the capsid protein of the PVY group viruses is primarily responsible for its anomalous electrophoretic behavior during SDS-PAGE.

Hiebert and McDonald (14) recently have shown that the condition of the capsid protein in turnip mosaic virus has a distinct effect on virus properties such as buoyant density, sedimentation rate, and serological reaction. Because we were unable to modify the observed capsid protein heterogeneity of DMV, we have not examined this effect on DMV properties. Therefore, the serological relationships presented for DMV isolates in this report must be viewed with some caution.

Dasheen mosaic was the only virus of aroids encountered in this study. All three DMV isolates were collected from widely separated sources (i.e., Florida, Fiji, and Egypt), but nevertheless had similar properties. Moreover, all three isolates proved to be closely related

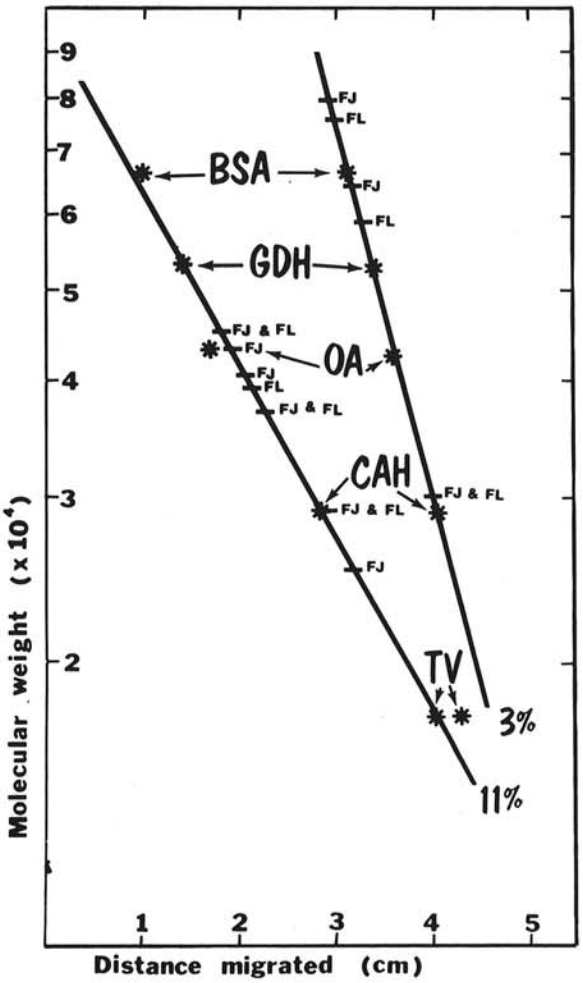

Fig. 5. Molecular weight determination of dasheen mosaic virus (DMV) isolates DMV-FL and DMV-FJ in 3 and 11% polyacrylamide gel slabs containing 0.1% sodium dodecyl sulfate and sodium phosphate buffer (pH 7.2). Marker proteins are bovine serum albumin (BSA), glutamate dehydrogenase (GDH), ovalbumin (OA), carbonic anhydrase (CAH), and tobacco mosaic virus coat protein (TV).

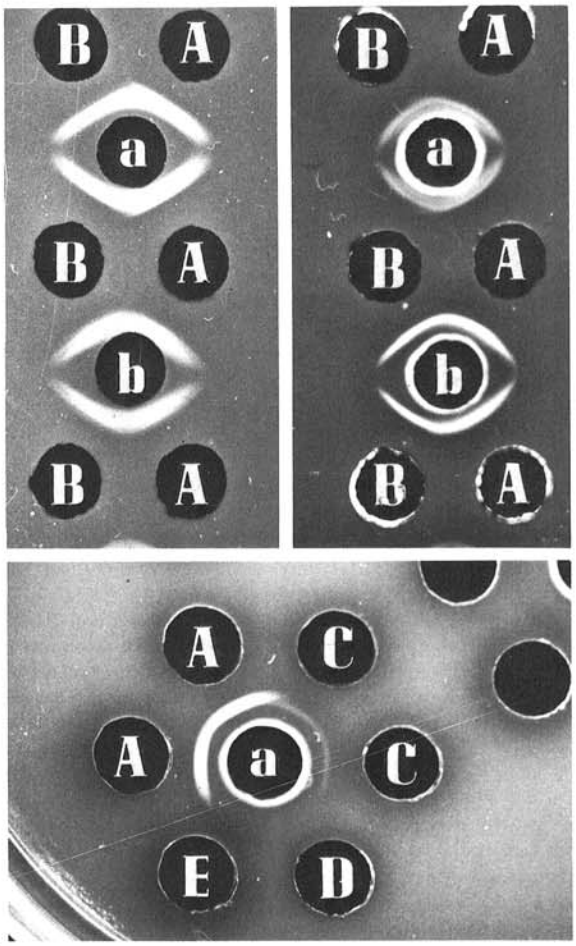

Fig. 6. Serological reactions of dasheen mosaic virus (DMV) in Ouchterlony agar-gel double diffusion tests. (Top left) The diffusion medium lacked sodium dodecyl sulfate, and the antigens were degraded in 2.5% pyrrolidine. (Top right and bottom) The diffusion medium contained 0.5% sodium dodecyl sulfate, and nondegraded antigens were tested. The wells identified by lower-case letters contained antisera prepared to isolates DMV-FL (a) and DMV-FJ (b). Wells identified by capital letters contained the following antigens: (A) DMV-FL, (B) DMV-FJ, (C) blackeye cowpea mosaic virus, (D) healthy cowpea, and (E) healthy *Philodendron selloum*.

serologically. The DMV-FJ isolate differed slightly from DMV-FL and DMV-E in symptoms induced in *P. selloum*. The DMV-FJ isolate also differed from DMV-FL in electrophoretic patterns in the SDS-PAGE system. It therefore is not surprising that DMV-FL appears to have an antigenic determinant not present in DMV-FJ on the basis of unilateral spur reactions observed in immunodiffusion tests. Similar differences have been noted between isolates of other viruses and are judged insufficient to consider DMV-FL and DMV-FJ as distinct viruses. The severe symptoms noted for DMV-FJ in MuMu taro is presumed to reflect a cultivar response of this cultivar to DMV.

Specific antisera to DMV isolates may have special significance, considering several factors which limit the study of this virus. Except for the possible susceptibility of *Tetragonia expansa* to DMV (7), which we have been unable to confirm, this virus appears to be restricted to members of the Araceae, and virus-free plants of such aroids as caladiums, *Colocasia* spp., dieffenbachia, and *Xanthosoma* spp. are not generally available for testing (2, 10, 12, 22).

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