Identification of Three Serotypes of Sowbane Mosaic Virus

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ABSTRACT

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No serological differences have been reported previously among isolates of sowbane mosaic virus (SoMV) described from several countries. In comparative immunodiffusion tests using a North American and two Moroccan SoMV isolates and their respective antisera, three distinct serotypes were defined. The two Moroccan SoMV serotypes did not differ in the molecular weights of their capsid protein subunits (32 kDa), and only minor biological differences between the three SoMV isolates were detected by using *Atriplex hortensis*, *A. semibacata*, *Chenopodium album*, *C. amaranticolor*, and *C. quinoa* as differential hosts.

Sowbane mosaic virus (SoMV), a member of the sobemovirus group, was first described in 1958 (7). It is distributed worldwide and has been reported to infect 24 plant species in five families (10). The virus is seed-transmitted in *Chenopodium* spp., which are widely used as virus indicator plants, and contamination by seedborne SoMV has sometimes led to erroneous results in plant virus research (4,10). No serological relationship to other viruses has been reported, and no serological differences have been found between previously described isolates of SoMV (1,2,4,6,10). The present paper identifies serological variability among isolates of SoMV from Morocco and the United States.

MATERIALS AND METHODS

Source of virus isolates. Three isolates of SoMV, designated SoMV-A2, SoMV-M1, and SoMV-M2, were used in this study. Isolate SoMV-A2 (ATCC PV 109) was obtained from the American Type Culture Collection. Isolate SoMV-M1 was isolated from a greenhouse plant of *Chenopodium quinoa* Willd. in Rabat, Morocco. Isolate SoMV-M2 was isolated from *C. murale* L. in the Souss Valley region of Morocco. The virus isolates were maintained in *C. quinoa* plants grown from virus-free seed.

Test plant reaction. Five seedlings of each test plant (as listed in Table 1) were mechanically inoculated with each isolate at the six-leaf stage. Inoculated plants and uninoculated controls were kept in a greenhouse and observed daily for 4 wk. Inoculated plants not showing symptoms were tested by agar gel immunodiffusion against SoMV antiserum to check for symptomless infection.

Purification. The SoMV isolates were purified by triturating frozen infected leaf tissue of *C. quinoa* 1:1 (w/v) in 0.5 M phosphate buffer, pH 7.0, containing 2% 2-mercaptoethanol and squeezing the homogenate through cheesecloth. Extracted juice was clarified by emulsifying with an equal volume of a 1:1 chloroform-butanol mixture, followed by centrifugation at 9,200 *g* for 10 min. The virus was precipitated from the aqueous phase with 8% polyethyleneglycol 8000 and 3% NaCl and collected by low-speed centrifugation. The precipitate was resuspended in 0.1 M phosphate buffer, pH 7.0, concentrated by centrifugation (78,600 *g* for 120 min), and resuspended in a small volume of the same buffer. This suspension was layered on a 10–40% linear-sucrose gradient and ultracentrifuged at 83,100 *g* for 150 min. Gradients were fractionated, and virus-containing fractions were collected and concentrated by centrifugation at 78,600 *g* for 2 hr. Virus was resuspended, centrifuged on a second 10–40% sucrose gradient, and stored frozen in 0.1 M phosphate buffer, pH 7.0.

Serology. Antisera against SoMV-A2, SoMV-M1, and SoMV-M2 were produced in rabbits by four intravenous injections of purified virus (2 mg each) administered during a 5-day period. Rabbits were exsanguinated 1 wk after receiving the last injection. A fourth antiserum was obtained from the American Type Culture Collection (ATCC PVAS 109a and herein termed AS-SoMV-A1). All serological tests were performed in 0.9% agarose containing 0.02% sodium azide in distilled water by using patterns of six wells around a central well. In intragel absorption tests, antisera wells were precharged with the absorbing antigens 24 hr before receiving antisera. Antigens, consisting of purified virus, were placed in peripheral wells so that different antigens were contiguous. The

TABLE 1. Reactions of *Chenopodiaceous* species to mechanical inoculation with three sowbane mosaic virus (SoMV) isolates

Test plant	SoMV isolate		
	SoMV-A2	SoMV-M1	SoMV-M2
Atriplex hortensis L.	CLL, SS[a]	SS	CLL, MS
A. semibacata Guss.	···	MS	–
Beta vulgaris L. cv. Blond Frisee	MS	MS	MS
Chenopodium album L.	···	CLL	CLL, MS
C. amaranticolor Coste & Reyne	···	CLL	–
C. quinoa Willd.	CLL, SS	CLL, SS	CLL, SS
Spinacea oleracea L.	···	SS	SS

[a] CLL = Chlorotic local lesions. MS = Mild systemic mosaic. SS = Severe systemic mosaic. – = No infection. ··· = Not tested.

TABLE 2. Possible antigenic determinants of three serotypes of SoMV determined by immunodiffusion and intragel absorption tests

Antigen	Antigenic determinants
A2	ABCD
M1	ABEF
M2	ACFG

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sixth well was charged with undiluted sap from uninoculated *C. quinoa*.

Protein subunit molecular-weight determination. The capsid protein molecular weights of the two Moroccan SoMV serotypes were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 10% polyacrylamide slab gels by using a continuous phosphate buffer system (9). Molecular weight markers were: phosphorylase-b (92.5 kDa), albumin (66.2 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), and lactalbumin (14.8 kDa). After electrophoresis at 5OmA for 8 hr, gels were stained in Coomassie blue R-250 in methanol-acetic acid-water and destained in methanol-acetic acid-water.

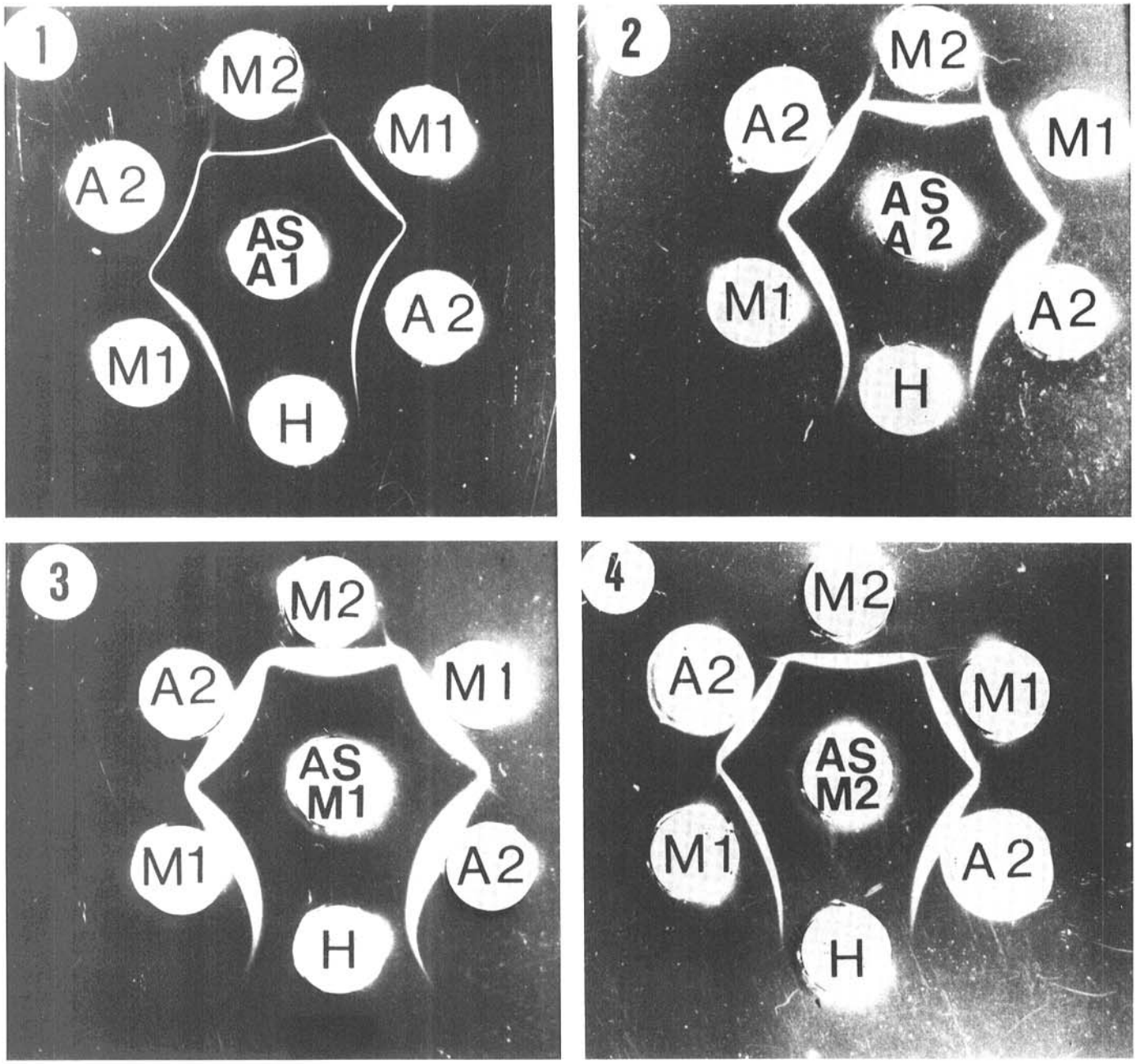
RESULTS AND DISCUSSION

Test plant reaction. Results (Table 1) demonstrated that the three isolates of SoMV could be differentiated on *Atriplex hortensis*. *A. semibacata*, *C. album*, and *C. amaranticolor* differentiate between isolates SoMV-M1 and SoMV-M2.

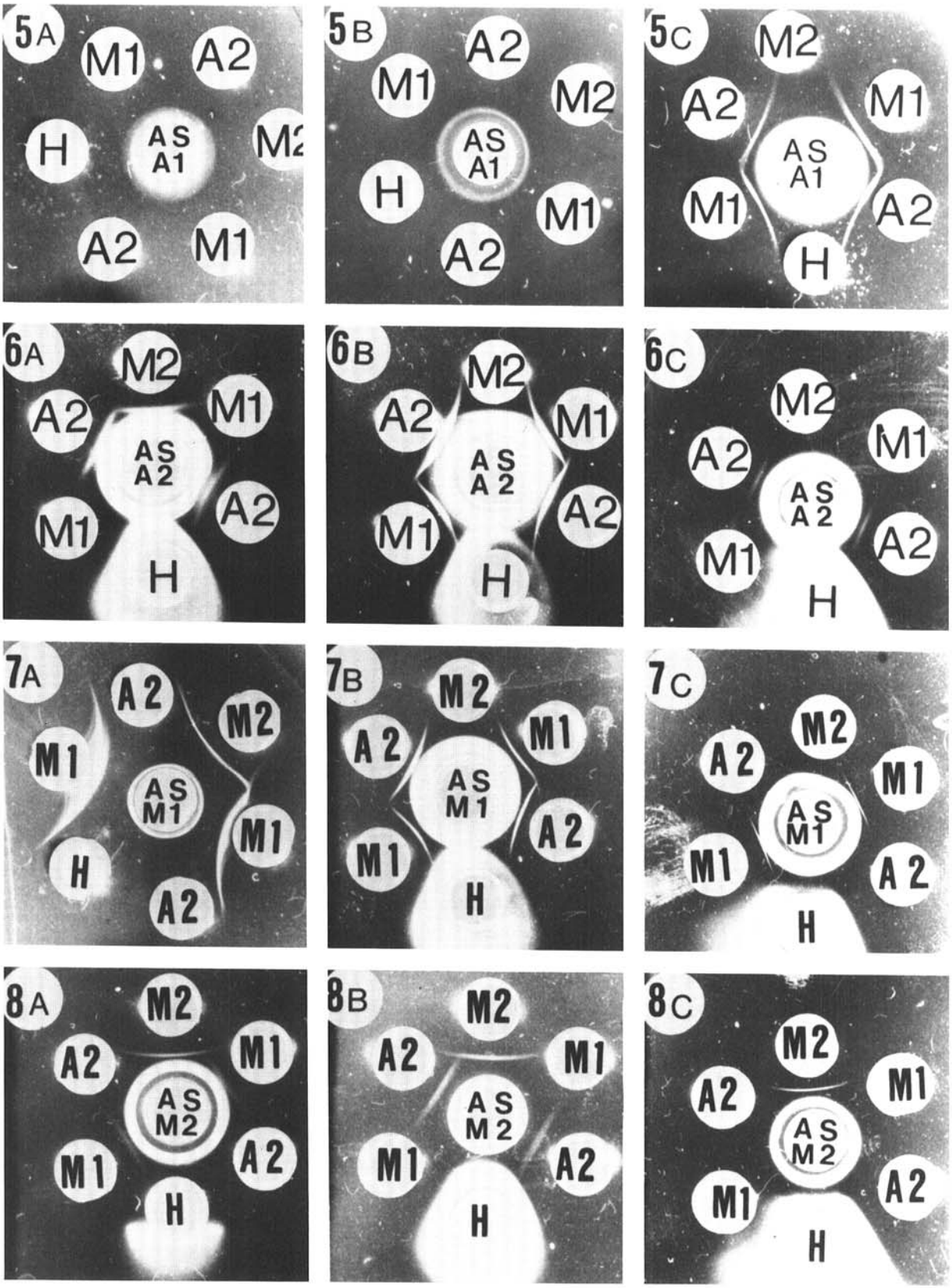
Purification. Virus suspensions of the three isolates had a UV absorption spectrum typical of nucleoproteins, with an $A_{260/280nm}$ of 1.5 (not corrected for light scattering), the same as the value reported previously for SoMV (6). Yields were in excess of 150 mg of virus per kilogram of fresh tissue.

Protein subunit molecular weight determination. Both Moroccan SoMV isolates had a single capsid protein of approximately 32 kDa. This value is in good agreement with that reported for the North American isolate of SoMV (31 kDa) (5).

Serology. Results demonstrated that all isolates are closely related to each other. All homologous and heterologous combinations reacted to dilution end points of 1:256 and 1:128, respectively. However, spur formation in immunodiffusion tests (Figs. 1–4) suggested that each isolate was serologically distinct.



Figs. 1–4. Reactions of an American and of two Moroccan SoMV isolates in homologous and heterologous combinations with their respective antisera and with American Type Culture Collection (ATCC) SoMV antiserum PVAS 109a. A2 = ATCC SoMV isolate PV 109. M1 = Moroccan SoMV isolate from *Chenopodium quinoa*. M2 = Moroccan SoMV isolate from *C. murale*. H = undiluted leaf sap from healthy *C. quinoa*. AS A1 = ATCC SoMV antiserum PVAS 109a. AS A2 = antiserum to ATCC SoMV isolate PV 109. AS M1 = antiserum to Moroccan SoMV isolate M1. AS M2 = antiserum to Moroccan SoMV isolate M2.



Figs. 5A–8C. Reactions of an American and two Moroccan SoMV isolates in homologous and heterologous combinations with their respective antisera and with ATCC SoMV antiserum PVAS 109a after intragel absorption with the SoMV antigens singly or in combinations. A2 = ATCC SoMV isolate PV 109. M1 = Moroccan SoMV isolate M1 from *Chenopodium quinoa*. M2 = Moroccan SoMV isolate M2 from *C. murale*. H = undiluted leaf sap from healthy *C. quinoa*. AS A1 = ATCC SoMV antiserum PVAS 109a. AS A2 = antiserum to ATCC SoMV isolate PV 109. AS M1 = antiserum to Moroccan SoMV isolate M1. AS M2 = antiserum to Moroccan SoMV isolate M2. Figures show reactions of SoMV isolates A2, M1, and M2 with: **5A,** ATCC PVAS 109a after intragel absorption with SoMV isolate A2 (ATCC PV 109); **5B,** ATCC PVAS 109a after intragel absorption with SoMV isolate M1; **5C,** PVAS 109a after intragel absorption with SoMV isolate M2; **6A,** AS A2 after intragel absorption with isolate M1; **6B,** AS A2 after intragel absorption with isolate M2; **6C,** AS A2 after intragel absorption with isolates M1 and M2; **7A,** AS M1 after intragel absorption with SoMV isolate A2; **7B,** AS M1 after intragel absorption with SoMV isolate M2; **7C,** AS M1 after intragel absorption with SoMV isolates A2 and M2; **8A,** AS M2 after intragel absorption with SoMV isolate A2; **8B,** AS M2 after intragel absorption with SoMV isolate M1; and **8C,** AS M2 after intragel absorption with SoMV isolates A2 and M1.

Differences among these serotypes were more evidently demonstrated by intragel absorption tests (Figs. 5–8).

Serological analysis. The appearance of a strong precipitin line between all antisera and all isolates (Figs. 1–4) revealed the existence of a common antigenic determinant designated here as A. Spur formation between isolate A2 and isolates M1 and M2 and double spurs existing between these two latter isolates when tested against antiserum A2 (Fig. 2) suggested the presence of two antigenic determinants, designated B and C, common to isolates A2 and M1 and to A2 and M2, respectively. This result was confirmed by intragel absorption tests (Fig. 6A and B) in which antiserum A2 was absorbed with isolates M1 and M2, respectively. The precipitin line formed between isolate A2 and its homologous antiserum absorbed with isolates M1 and M2 (Fig. 6C) demonstrated that this isolate has a distinctive antigenic determinant designated here as D. This interpretation suggests spur formation should occur between isolates A2 and M2 in Figure 6A. This was not observed and may be due to a low concentration of one of the reactants. Spurs formed between isolates M1 and A2, M2 and M1, and A2 and M2 when tested against antiserum M1 (Fig. 3) confirmed the existence of an antigenic determinant common to isolates A2 and M1 and suggested the existence of an antigenic determinant specific to isolate M1. This result was confirmed by an intragel absorption test in which a preciptin line was formed between isolate M1 and its own antiserum absorbed with isolates A2 and M2 (Fig. 7C). This determinant was designated as E.

Precipitin lines formed between isolates M1 and M2 and antiserum M1 when isolate A2 was used as the absorbing antigen (Fig. 7A) indicated that isolates M1 and M2 have a common antigenic determinant designated as F. Presence of an antigenic determinant specific to isolate M2, designated here as G, is shown by spur formation between isolates M2 and M1 using antiserum M2 absorbed with isolate A2 (Fig. 8A) and between isolates M2 and A2 using antiserum M2 absorbed with isolate M1 (Fig. 8B). This is in agreement with the reaction between isolate M2 using antiserum M2 absorbed with isolates A2 and M1 (Fig. 8C) and spur formation between isolates M2 and M1 and M2 and A2 using antiserum M2 (Fig. 4). Comparison of Figures 5B and C with 6A and B and of Figures 1 and 2 suggests that antisera A1 and A2 contain different populations of antibodies. These results may be caused by a differential response of different animals to the same antigen (8) or alternatively, the existence of an additional antigenic variant of the virus.

The data we report demonstrate that the three isolates A2, M1, and M2 of SoMV can be distinguished as serotypes. Seven unique antigenic determinants were detected in this study (Table 2). Other antigenic determinants may exist that were not detected because of the limited sensitivity of the techniques employed or may be present on previously undescribed isolates of this virus. Although the conformation of the three serotypes used under the conditions of these tests is unknown, recent evidence comparing the reaction of intact and dissociated barley yellow dwarf virus to polyclonal and monoclonal antibodies has demonstrated the relevance of antigen conformation to interpretation of serological tests (3).

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