Squash Mosaic Virus Variability: Review and Serological Comparisons of Six Biotypes

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ABSTRACT

Variants of squash mosaic virus were collected from cucurbit producing areas of the western hemisphere. There were six biotypes in the collection, based on symptomatology and host range. One isolate of each biotype was used to prepare antisera and all were cross-reacted serologically. Only two groups were distinguished on the basis of serological reactions. Group I isolates caused severe symptoms on cantaloupes and most mild symptoms on pumpkins (initially with ringspots). Some members of Group I infected watermelon. No members of Group II infected watermelon, but all produced mild symptoms on cantaloupe and severe effects on pumpkin. With this information it was possible to analyze previous reports of squash mosaic and to assign many of the causal viruses to a serological group.

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Additional key words: intragel absorption, symptomatology, host range.

Squash mosaic virus (SMV) was probably first described by McClintock (22) in 1916. Although he called the virus "cucumber mosaic" it seems likely, in view of the high rate of seed transmission demonstrated with cucumber, that he was actually working with a strain of SMV. Kendrick, however, is

TABLE 1. Isolates of squash mosaic virus used in this study[a]

Isolate	Geographical origin	Characteristic symptoms on		
		Cantaloupe	Pumpkin	Watermelon
IA[b]	Yuma, Arizona	VB SM[c]	RS MM	SS
IB	Yuma, Arizona	VB SM	RS MM	SS
IC	Yuma, Arizona	VB SM	RS MM	SS
ID	Yuma, Arizona	VB SM	RS MM	SS
IE	Florida	VB SM	RS SD	Neg
IF[b]	Puerto Rico	VB SM	RS SD	Neg
IG[b]	California	VB SM	RS MM	Neg
IH[b]	Colorado	VB SM	RS MM	LL
II	Colorado	VB SM	RS MM	LL
IIA[b]	Wisconsin, severe	MM	SD SM	Neg
IIB	California	MM	SD SM	Neg
IIC	South Dakota	MM	SD SM	Neg
IID[b]	Wisconsin, mild	MM	SD SM	Neg

[a] The isolates are grouped according to information obtained in this investigation.
[b] Used in serological tests.
[c] Symptoms: SM = severe mosaic; MM = mild mosaic; VB = vein banding; LL = local lesions; Neg = no infection; SD = severe distortion; RS = ringspot; SS = severe stunt.

generally credited with the first report of SMV in 1934 (15). During the next 20 years, more reports of seed-transmitted cucurbit mosaic were published (10, 11, 12, 19, 23) which included the first detailed characterization of the virus in 1956 by Freitag (13). He reported that the virus was stable with respect to temperature, dilution, and aging; and that the host range was restricted to cucurbits with the exception of some genera from the Leguminosae, Umbelliferae, and Hydrophyllaceae. Of particular importance to this characterization was the fact that SMV did not infect watermelon (*Citrullus vulgaris* Schrad.), although it did infect citron or preserving melon (*Citrullus vulgaris* Schrad. var. *citroides* Bailey). For the next ten years this characteristic (lack of infection of watermelon) was used diagnostically in part to distinguish SMV from other viruses that infected cucurbits. In 1956, Lindberg et al. (19) distinguished between squash mosaic and melon mosaic isolates, serologically grouping viruses that previously had a variety of labels under two names. No indication was given in their paper that any of these SMV isolates infected watermelon locally or systemically. Grogan et al., 1959 (14), pointed out that SMV isolates they had obtained from muskmelon seedlings caused local lesions on watermelon as well as ringspots on pumpkin. In 1965, isolates of SMV which infected watermelon systemically were obtained by Nelson et al. (25) from cantaloupes.

There is some variation in reports of temperature of inactivation, dilution end point, and longevity in vitro of individual isolates but these data can be summarized thus: (i) SMV isolates are completely inactivated after 10 minutes of exposure at 65 C in vitro; (ii) sap from SMV-infected plants must be diluted 10^5 - 10^6 times before all infectivity is lost; (iii) strains of SMV will resist complete inactivation in crude sap at room temperature for periods in excess of 1 month. None of these properties is useful for distinguishing between strains of squash mosaic virus. They do, however, identify squash mosaic as a very stable virus in vitro, which distinguishes it from several other cucurbit viruses.

Two separate antigenic types have been shown to exist among variants of squash mosaic virus (16). While serological specificity was associated with host range; e.g., watermelon and nonwatermelon-infecting strains, the acquisition of additional SMV isolates has shown that this association is not consistent. This present work more fully assesses the type and degree of variability found in squash mosaic virus, and relates this information to previous reports of cucurbit viruses.

MATERIALS AND METHODS.—Virus isolates were obtained and investigated to fully determine the range of variability in squash mosaic strains. Attempts were made to obtain virus isolates investigated by Aycock (2), Anderson (1), Lindberg et al. (19), Stoner (29), Freitag (13), Rader (27), and Perez (26). We obtained the following: Anderson's typical muskmelon mosaic (from R. W. Fulton - Wisconsin), pumpkin mosaic (29), Perez' melon mosaic virus (26), Freitag SMV (13), Wisconsin severe and mild squash mosaic strains (19), and several watermelon infecting isolates from Arizona (25). In 1970, we obtained an isolate from R. N. Campbell isolated from honeydew melon (*personal communication*). Also in 1970, two isolates were obtained from seedlings grown from seed harvested in 1968 in Colorado.

Transmission to watermelon (*Citrullus vulgaris* Schrad.) was attempted with all isolates. Watermelon infection was considered positive only if virus was transmitted back to pumpkin (*Cucurbita pepo* L.). Repeated attempts were made with those which initially failed to infect watermelon. Vigorously growing plants were observed for symptoms for 2-4 weeks following inoculation of their cotyledons.

Serological characterization was done with isolates representing all six biotypes collected (Table 1).

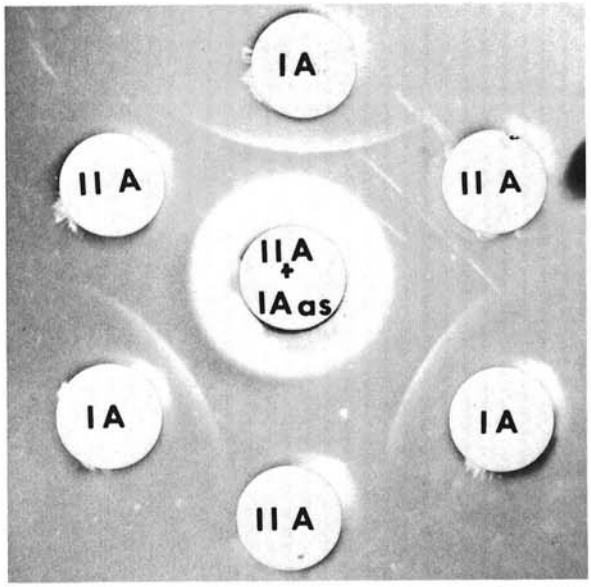


Fig. 1. Intragel absorption. Absorption of antiserum IA by IIA antigen and subsequent reaction of IA antigen with heterologous anitbodies are indicated. The alternate wells where no reaction has taken place contain IIA antigen, confirming that cross absorption was complete. The reciprocal test was identical.

Isolates from group II were multiplied in pumpkin, whereas those of group I were multiplied in cantaloupe. The most convenient and effective purification scheme consisted of grinding infected tissue in a Waring Blendor with two volumes of distilled water. After filtration of the extract through cheesecloth, the pH was adjusted to 5.0 with 10% acetic acid. The preparation was stirred 30-60 min at 4 C, then clarified, and sufficient polyethylene glycol (PEG) was added to make a solution of 8%. This solution was stirred for 30 min and then centrifuged at 10,000 *g* for 20 min. The pellet, which contained the virus, was resuspended in 0.1 M pH 7.0 phosphate buffer and the PEG precipitation repeated one or more times to concentrate the virus. The final step in purification, before immunization of the rabbits, was density-gradient centrifugation. A linear gradient of 100-400 mg sucrose/ml was used in 2.54 × 8.89 cm (1 × 3.5 in.) cellulose nitrate tubes in the Beckman SW 27 rotor. This rotor was run at 27,000 rpm for 90 min. The three bands characteristic of all isolates of SMV were collected, pooled, and after dialysis for 24 hr against 50 volumes of 0.1 M pH 7.0 phosphate buffer, used as the immunizing antigen. During a period of 5 weeks, rabbits were given five intravenous injections of 2-4 mg of virus and one intramuscular injection of 4-6 mg in Freund's incomplete adjuvant. One week after the last injection the rabbits were bled by non-terminal cardiac puncture. Several additional bleedings were made during this week and all were pooled.

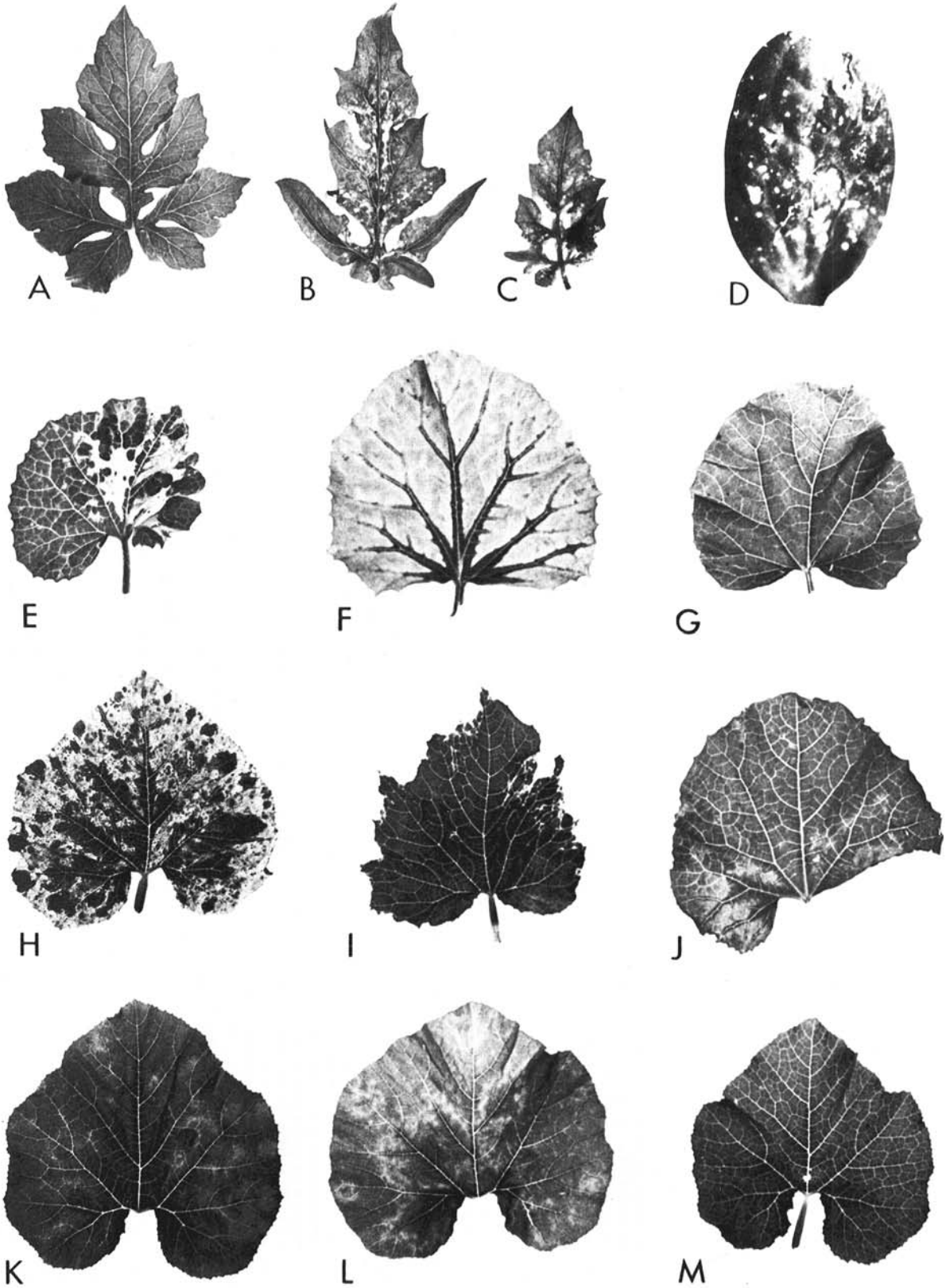
Two types of serological tests were used. All antigens were tested with all other antisera in agar double-diffusion tests to encourage spur formation by heterologous antibodies. Intragel absorptions were also run in all possible combinations by placing antigen in the center well 12-24 hr prior to the introduction of the heterologous antiserum. In the outer wells, the homologous and heterologous antigens were alternated (Fig. 1).

RESULTS.—*Serology.*—The two types of serological tests conducted produced the same results. All six antisera when reacted against alternating homologous and heterologous antigens in outer wells of gel diffusion plates produced spurs only when an antiserum of group I was reacted in this manner with an antigen of group II and vice versa. Each member of group I reacted as a homologous antigen with all antisera of group I, as did the two members of group II with each other. Intragel absorption tests led to the same conclusion. After cross-absorption, heterologous bands were produced only when an antiserum of group I was absorbed against an antigen of group II and then reacted with a group I antigen (Fig. 2 illustrates this), and vice versa.

Host range.—Members of both groups infect most common cucurbits and members of some other plant families (13, 25). Several members of group I infect watermelon, but none of group II infects this host. Fifteen varieties of watermelon were tested for susceptibility to SMV isolates. No varietal differences in susceptibility to SMV strains were detected.

Symptomatology.—Differences in symptoms between the two groups are best expressed in pumpkin and cantaloupe. On pumpkin, infection by isolates of group I results initially in the formation of chlorotic ringspots. These soon fade and subsequent growth shows little more than a mild mottle. There are two exceptions within group I. The Florida (1) and Puerto Rican (26) isolates cause symptoms on pumpkins very similar to those for group II isolates except for the initial formation of ringspots which is a group I characteristic. On cantaloupe, all members of group I cause virtually identical symptoms. Characteristic symptoms are interveinal chlorosis resulting in green bands along veins; leaf distortion and prominent serrations on leaf margin are also typical. Symptoms may vary from leaf to leaf of a single plant. Members of group II infect cantaloupe and cause a mild mottle which may disappear entirely with time. The reaction of pumpkin to members of group II is severe, typified by mosaic and distortion of normal leaf tissues.

Fig. 2. Symptoms which aid in differentiating between the two groups of squash mosaic virus. **A)** Healthy watermelon; **B, C)** watermelon infected with IA; **D)** watermelon cotyledon with local lesions of IH; **E, F)** cantaloupe infected with IA; **G)** healthy cantaloupe; **H, I)** pumpkin infected with IIA; **J)** cantaloupe infected with IIA; **K, L)** pumpkin infected with IA; **M)** healthy pumpkin.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Two symptom types on watermelon caused by members of group I include: (i) systemic chlorotic and necrotic spotting and severe stunt, and (ii) a local necrotic spotting only.

All these symptom types are illustrated in Fig. 2.

DISCUSSION.—The purpose of this report was to explore in greater depth than has been done previously, the extent of variability of squash mosaic virus strains. All isolates were obtained from the western hemisphere. Three basic conclusions were reached as a result of this work:

(i) Only two basic strains of squash mosaic virus

TABLE 2. Chronological listing of reports of cucurbit viruses which probably involved squash mosaic virus (SMV)[a]

Author	Virus name	Original host	Symptoms on: Muskmelon	Symptoms on: Watermelon	Symptoms on: Squash pumpkin	Seed transmission (%)	Serological group	Basis for group designation[c]
McClintock 1916 (22)	Cucumber mosaic	*Cucumis sativus* L.	NI[b]	NI	NI	37	I	High seed transmissions *Cucumis* spp.
Kendrick 1934 (15)	Cucurbit mosaic	*Cucumis melo* L.	SM	NI	NI	2.13	I	Symptomatology original host Seed transmission
Mahoney 1935 (20)	Mosaic	*Cucumis melo* L.	NI	NI	NI	24 (Avg)	I	Symptomatology original host Seed transmission
Freitag 1941 (11)	Cucurbit ring mosaic	*Cucurbita* spp.	NI	NI	RS	NI	I	Symptomatology
Freitag 1941 (11)	Squash mosaic	*Cucurbita* spp.	NI	NI	SD	NI	II	Symptomatology
Middleton 1944 (23)	Squash mosaic	*Cucurbita moshata* L.	NI	NI	SM SD	5-2.0 (Avg 0.3)	II	Symptomatology
Rader et al. 1947 (27)	Muskmelon mosaic	*Cucumis melo* L.	VB MM	NI	NI	6-93	I	Symptomatology High seed transmission in muskmelon
Aycock 1951 (2)	Cantaloupe mosaic	*Cucumis melo* L.	VB MM	LL	RS	NI	I	Symptomatology Host range
Anderson 1954 (1)	Muskmelon mosaic typical strain	*Cucumis melo* L.	VB MM	Neg	RS MM	NI	I	Serology
Anderson 1954 (1)	Muskmelon mosaic latent strain	*Melothria pendula* L.	MM	NI	RS MM	NI	I	Symptomatology
Freitag[d] 1956 (13)	Squash mosaic	*Cucurbita* spp.	MM	Neg	SM SD	NI	II	Serology
Lindberg[d] et al. 1956 (19)	Mild squash mosaic	*Echinocystis* spp. (wild cuc.)	MM	Neg	MM	NI	II	Serology
Lindberg[d] et al. 1956 (19)	Severe squash mosaic	*Echinocystis lobata* (wild cuc.)	MM	Neg	SD	.4	II	Serology
Grogan et al. 1959 (14)	Squash mosaic 10 isol.	*Cucumis melo* L.	MM	LL	RS	.3-20 (Avg 3.5)	I	Host range Symptomatology
Cohen & Nitzany 1963 (4)	Squash mosaic	*Ecbalium elaterium* (L.) Rich.	NI	NI	NI	NI	?	Inadequate characterization
Perez[d] 1963 (26)	Muskmelon mosaic	*Cucumis melo* L.	SM	Neg	RS	.33	I	Serology
Stoner[d] 1963 (29)	Pumpkin mosaic	*Cucurbita pepo* L.	MM	Neg	SD	NI	II	Serology
Nelson[d] et al. 1965 (25)	Watermelon stunt	*Cucumis melo* L.	VB MM	LL SS	RS MM	NI	I	Serology
Demski 1969 (5)	Squash mosaic	*Cucumis melo* L.	NI	LL	RS	NI	I	Symptomatology Host range

[a] Virus reports are placed in serological groups based on reasons indicated.

[b] Symptoms: SM = severe mosaic; MM = mild mosaic; VB = vein banding; LL = local lesions; Neg = no infection; SD = severe distortion; RS = ringspot; SS = severe stunt; NI = not investigated.

[c] Where serological tests were possible, other characteristics not listed.

[d] Virus isolates investigated by the authors.

exist. Although in group I there is some intrastrain variability in host range and symptom type, group II shows no such variability. Even . though isolate IID was once labeled "mild" SMV, that descriptor in one way or other seems to have changed over the years.

(ii) The most prevalent isolates from cucurbits are members of group I. There is no published information to suggest that any members of group II have ever been isolated from *Cucumis* spp., whereas group I members have been isolated from both *Cucumis* and *Cucurbita* spp.

(iii) The lack of group II adaptability to *Cucumis* seems to be in part a result of the lack of seed transmissibility of group II members in species of this genus (24).

Differential seed transmissibility appears to have had profound effects on the ecology of these two groups.

Despite the fact that members of group I are the most numerous, most chemical and physical studies have been done with members of group II (3, 7, 21, 28, 30).

The application of this work is illustrated in Table 2 where previous reports of cucurbit virus diseases believed to be squash mosaic were analyzed. This analysis resulted in the placement of most viruses reported into group I or II based upon the information provided in each report.

The only report of SMV outside the western hemisphere that is substantiated is from Israel. Reports of squash mosaic have been made from Europe but the reported characteristics make such claims questionable (8, 9). In addition to mainland U.S. and Puerto Rico (26) SMV has been isolated in Venezuela (17, 18) but could not be assigned to strain on the basis of published characteristics.

Seed transmission of cucumber mosaic virus has been reported several times over the years (6, 22, 31). We feel that in each of these cases squash mosaic virus was involved because the identity of CMV had not been properly confirmed. One of the most significant cases is that by Doolittle & Walker (6) who in 1925 reported that an average of 9% of the progeny seedlings of seed of mosaic-infected wild cucumber (*Echinocystis lobata*), collected in the wild in the upper midwest, were infected with CMV. That this was CMV was not properly confirmed by techniques available at that time. Furthermore, the observations made by the same author that cucumber beetles transmitted the virus from wild to cultivated cucumbers, is additional evidence that supports the idea that it was SMV that was present in wild cucumber. Nevertheless, for 50 years it has been assumed that such alleged seed transmission of CMV in wild cucumber was an important overwintering mechanism of this virus and an important source of virus for infection of cucumbers in the spring (32). Twenty-eight years later in 1953 (19) two isolates of squash mosaic virus were obtained from wild cucumber in Wisconsin. Because of the propensity of SMV (but not CMV) to be seed-transmitted in cucurbit species, it seems likely that this virus is the same as originally observed by Doolittle in seedlings of wild cucumber and that indeed the seed of *Echinocystis lobata* does not serve as an overwintering mechanism for CMV. Similarly a recent report of seed transmission of CMV from the same host in Czechoslovakia (31) probably involved SMV and not CMV as was reported. In this case also, proper identification procedures were not described to establish the virus as CMV. If, as we suspect, the virus was SMV and not CMV, that report would have been the first record of the isolation of SMV in Europe.

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