Characterization and Variability of Strains of Southern Bean Mosaic Virus

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ABSTRACT

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A strain of southern bean mosaic virus (SBMV) is described that infects bean cultivars with resistance to the type strain of this virus. This strain gave rise to three additional strains. The host ranges, physical properties, sedimentation coefficients, serology, nucleic acid, protein, and beetle transmission characteristics are compared with the SBMV-type strain. In most characteristics all strains are similar. Serology distinguishes SBMV-type from the other four. All five can be distinguished on the basis of host range reaction.

Additional key words: Phaseolus vulgaris, susceptibility, host passage effects.

Several strains of southern bean mosaic virus (SBMV) have been described. Southern bean mosaic virus (type strain) (14), severe bean mosaic virus (SvBMV) (12), cowpea southern bean mosaic virus (CP-SBMV) (10), and a cowpea strain from Ghana (SBMV-GH) (5) are serologically related, but not identical. They possess similar chemical, physical, and some biological characteristics (3). They differ, however, in host range and electrophoretic mobility as well as serology.

A strain of SBMV (designated SBMV-A) that systemically infected bean (*Phaseolus vulgaris* L.) cultivars with resistance to SBMV-type was isolated from plant material that had been desiccated and stored for approximately 30 yr. Records of the origin of the original sample of SBMV-A have been lost, but it was in a collection of isolates of bean common mosaic virus (BCMV) from various locations in the United States. The sample contained both SBMV and BCMV.

Three other strains (designated SBMV-B, C, and D) that differ in host reaction were also isolated from plants inoculated with SBMV-A. This study was done to characterize these strains of SBMV, to describe their variability, and to compare them with the type strain.

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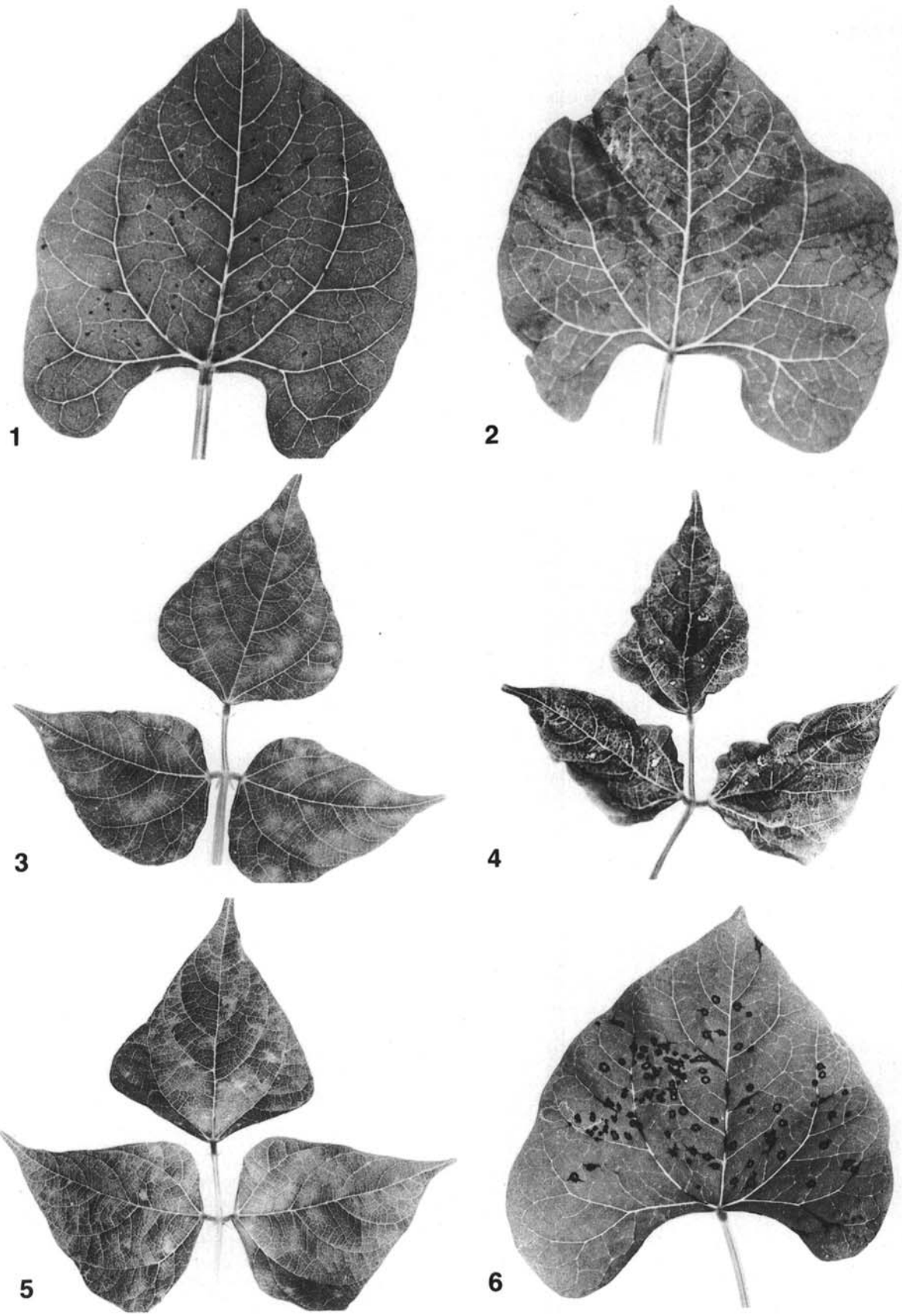
MATERIALS AND METHODS

The host reactions of 18 bean cultivars, as well as other legumes and nonlegumes, to SBMV-type and SBMV strains A, B, C, and D were tested. The symptoms were recorded and the presence of virus was determined by serology.

The physical properties were determined according to procedures described by Yerkes and Patino (12) by utilizing crude sap from systemically infected *P. vulgaris* 'Black Valentine,' which was also used as an assay host.

Virus was purified by using a modification of Steere's chloroform-butanol method (11). Sap from cultivar Black Valentine bean was extracted 2 wk after inoculation with 1.5 ml of 0.2 M sodium phosphate buffer, pH 7.2, containing 0.1 M ascorbic acid and 1 ml of chloroform-butanol (1:1) per gram of tissue. The extract was stored overnight at room temperature and centrifuged at 5,000 *g* for 10 min. The aqueous phase was then subjected to three alternate high-speed (80,000 *g* for 90 min for the first and 60 min for the second and third) and low-speed (5,000 *g* for 10 min) centrifugations. High-speed pellets were resuspended in 0.01 M sodium phosphate buffer, pH 7.2.

Purified virus preparations (3 mg/ml) in 0.01 M sodium phosphate buffer were centrifuged at 32,000 rpm in the An-D rotor in a Beckman analytical ultracentrifuge with Schlieren optics. Sedimentation coefficients were determined according to Markham's graphical method (8).



Figs. 1–6. Symptoms induced by strains A, B, C, and D of southern bean mosaic virus (SBMV) on two bean cultivars. **1,** Necrotic local lesions induced by SBMV-A (or SBMV-C) on inoculated primary leaf of cultivar Black Turtle Soup. **2,** Vein necrosis induced by SBMV-C (or SBMV-D) on an inoculated primary leaf of cultivar Pinto. **3,** Systemic mottle on Pinto induced by SBMV-A (or SBMV-B). **4,** Systemic leaf malformation and necrosis on Pinto induced by SBMV-C (or SBMV-D). **5,** Systemic mottle on Black Turtle Soup induced by SBMV-B (or SBMV-D or SBMV-type). **6,** Necrotic local lesions on inoculated primary leaf of Pinto induced by SBMV-type.

Antisera to SBMV-type, isolate A, and isolate B were produced in rabbits by four weekly, subcutaneous injections of 0.5-ml suspensions of virus (7.0 mg/ml) emulsified in 0.5 ml of Freund's incomplete adjuvant.

Purified SBMV-type, A, B, C, and D were reacted in various combinations with SBMV-type, A, and B antisera (titer > 1:500) in 1% Ionagar #2 by using the Ouchterlony double-diffusion method to determine relationships.

Immunoelectrophoresis was performed using purified virus as described by the Gelman Instrument Co. manual (2).

Molecular weights of the protein subunits were determined by electrophoresis on 7.5% acrylamide gels as described by Maizel (7). The protein standards used were myoglobin (mol wt 17,200 daltons), carbonyl anhydrase (mol wt 30,000 daltons), catalase (mol wt 60,000 daltons), and phosphorylase (mol wt 94,000 daltons). Molecular weights of the RNAs were estimated with 2.9% polyacrylamide gel electrophoresis by using Lane's method (6) and SBMV-type RNA (mol wt 1.4×10^6 daltons) as a standard (1). For examination in the electron microscope, purified virus was mixed with an equal volume of 2% phosphotungstic acid, adjusted to pH 6.5 with NaOH, and applied to a Formvar-coated grid.

In vector studies, single bean leaf beetles (*Cerotoma trifurcata* Forst.) and Mexican bean beetles (*Epilachna varivestis* Muls.) were given acquisition feedings of 24 hr on leaves of infected *P. vulgaris* 'Pinto' and 'Black Turtle Soup' bean plants. To test for transmission, beetles were fed on caged healthy Pinto and Black Turtle Soup beans for 24 hr. After 2 wk, sap from test plants was checked for virus by serology.

Seed transmission of SBMV was evaluated by growing Black Valentine bean plants infected with either SBMV-type or SBMV-A to maturity in the greenhouse. Mature seed were planted immediately, and the resulting plants were tested for virus.

RESULTS

Origin of strains. In host range studies, SBMV-A was found to vary and to produce three other distinct strains. The bean cultivar Black Turtle Soup (NSSL Accession 9251B) reacted with the production of necrotic local lesions when inoculated with SBMV-A. Occasionally, after 2 or 3 wk, systemic symptoms appeared in the trifoliolate leaves of Black Turtle Soup plants inoculated with SBMV-A. SBMV-B was isolated from these systemically infected plants. In plants of cultivar Pinto infected by SBMV-A, the trifoliolate leaves developed a systemic mottle with occasional necrotic lesions. From such lesions SBMV-C was isolated. Plants of cultivar Black Turtle Soup inoculated with SBMV-C typically developed necrotic local lesions. Some plants also developed a systemic mottle after 2 wk from which SBMV-D was isolated. Each strain acted in a consistent manner. The production of SBMV-B from SBMV-A and SBMV-D from SBMV-C were consistent features of these isolates. Repeated local lesion transfer of SBMV-A or SBMV-C on Black Turtle Soup bean failed to detect other strains and no strain could be selected that produced only necrotic local lesions on Black Turtle Soup without occasionally producing SBMV-B or SBMV-D in this host. The symptoms induced by these four strains and SBMV-type on plants of bean cultivars Pinto and Black Turtle Soup are shown in Figs. 1–6.

Host range. The reactions of 18 bean cultivars to five strains of SBMV are shown in Table 1. All bean cultivars that developed a systemic mottle showed this symptom 1 wk after inoculation. Cultivars that reacted with vein necrosis developed this symptom in the inoculated area 5 days after inoculation and systemic vein necrosis and leaf malformation later. Necrotic local lesions appeared 5 days after inoculation.

No infection was obtained when the following plants were mechanically inoculated: *Chenopodium quinoa* Willd.; *Cucumis sativus* L., 'Model'; *Glycine max* (L.) Merr. 'Lee,' 'Bragg,' and 'Forest'; *Dolichos lablab* L.; *Lupinus albus* L.; *Phaseolus aureus* Roxb.; *Vigna unguiculata* (L.) Walp., subsp. *unguiculata* 'Monarch,' 'Crimson,' 'California Blackeye No. 5,' 'Georgia 21,' 'Black,' and 'Chinese Red.'

Physical properties. No difference could be detected in the physical properties among the type strain and strains A, B, C, and D. In all cases, the dilution end point was greater than 10^{-6}, the thermal inactivation point was between 90–95 C, and the longevity in vitro was approximately 4 wk.

Analytical ultracentrifugation. Comparison of the sedimentation coefficients of SBMV-A, B, C, and D with SBMV-type resulted in similar S values which ranged from 109 to 115 S.

Serology. In Ouchterlony double diffusion tests, SBMV-type, and strains A, B, C, and D reacted with SBMV-type antiserum forming coalescing lines of precipitate without forming spurs. When SBMV-A or B antiserum was used, no spurs formed between SBMV-A, B, C, or D, but spurs formed with each of these strains and SBMV-type. All strains showed only one electrophoretic component that migrated the same distance toward the anode.

Nucleic acid. In polyacrylamide gel electrophoresis, the RNAs of all strains when compared with the RNA of the type strain migrated the same distance as single sharp bands, indicating that all have similar molecular weights ($\sim 1.4 \times 10^6$ daltons [1]).

Protein. Polyacrylamide gel electrophoresis showed that proteins of all strains migrated the same distance, indicating that each is a single component with a molecular weight of approximately 29,000 daltons (3).

TABLE 1. Reactions[a] of bean cultivars following mechanical inoculation of southern bean mosaic virus (SMBV)-type, and strains SBMV-A, SBMV-B, SBMV-C, and SMBV-D.

Bean cultivar	Virus strain				
	SBMV-type	SBMV-A	SBMV-B	SBMV-C	SBMV-D
Black Valentine	S	S	S	S	S
Black Turtle (Gurney)	S	S	S	S	S
Black Turtle Soup	S	LLn(S)	S	LLn(S)	S
Pencil Pod Black Wax	S	S	S	S	S
Blue Lake	S	S	S	S	S
Cherokee Wax	S	S	S	S	S
Dade	LLn	S	S	SVn	SVn
Dwarf Horti-cultural	S	S	S	S	S
Dutch Case Knife	LLn	S	S	SVn	SVn
Kentucky Wonder Brown	LLn	S	S	SVn	SVn
Kentucky Wonder White	LLn	S	S	SVn	SVn
MacCalsan #42	LLn	S	S	SVn	SVn
Pinto	LLn	S(Ln)	S	SVn	SVn
Rebel	S	S	S	S	S
Red Kidney	S	S	S	S	S
Topcrop	S	S	S	S	S
Viva Pink	LLn	S	S	SVn	SVn
White Half Runner	S	S	S	S	S

[a] S = systemic mottle; SVn = systemic vein necrosis and leaf malformation; LLn = necrotic local lesions; LLn(S) = necrotic local lesions and occasional systemic mottle; and S(Ln) = systemic mottle with occasional necrotic lesions.

TABLE 2. Beetle transmission of southern bean mosaic virus (SBMV)-type, SBMV-A, SBMV-B, SBMV-C, and SBMV-D

Beetle	Virus strain				
	SBMV-type[a]	SBMV-A[b]	SBMV-B[a]	SBMV-C[b]	SBMV-D[a]
Epilachna varivestis	33/35[c]	20/36	7/20	8/25	14/35
Cerotoma trifurcata	17/50	10/30	5/19	3/14	2/10

[a] Using bean cultivar Black Turtle Soup as acquisition and transmission host.

[b] Using bean cultivar Pinto as acquisition and transmission host.

[c] Ratio: (number of beetles that transmitted virus)/(number of beetles tested).

Electron microscopy. No difference in particle size or shape was found among the strains. The icosahedral particles ranged between 26 and 29 nm.

Beetle transmission. SBMV-type and strains A, B, C, and D were transmitted efficiently by the bean leaf beetle and the Mexican bean beetle (Table 2).

Seed transmission. With SBMV-type and SBMV-A low levels of seed transmission (5 and 3%, respectively) were detected.

DISCUSSION

The physical properties of strains SBMV-A, B, C, and D are similar. They differ only in the reaction of some bean cultivars to infection following mechanical inoculation. They differ from SBMV-type in serology as well as host reaction. The difference in host reaction is significant in this case, since bean cultivars with resistance to the type strain are susceptible to strains SBMV-A, B, C, and D. Cultivar Black Turtle Soup was the only one with resistance to SBMV-A and C, but symptoms caused by the variant strains B and D sometimes appeared after 2 wk when this cultivar was inoculated with A or C.

Resistance to systemic spread of a virus associated with the production of necrotic local lesions is often conferred by a single dominant gene (9). This is the case with SBMV-type and the bean cultivars that react with necrotic local lesions (13). The A, B, C, and D strains overcome that resistance. Cultivar Black Turtle Soup probably has a single dominant gene for resistance to SBMV-A and C but lacks a gene for resistance to SBMV-type, B, and D.

SBMV-A gave rise to SBMV-C when inoculated to Pinto beans. SBMV-A and SBMV-C gave rise to SBMV-B and SBMV-D, respectively, when inoculated to Black Turtle Soup beans. Mutation probably plays an important role in the variability of the virus.

Several facts confirm that this variability is actually occurring and that four different strains of SBMV were isolated. We were unable to obtain, by single local lesion transfers, an isolate of SBMV-A or C that caused only local lesions on leaves of Black Turtle Soup bean plants. The delayed appearance of systemic symptoms in some plants was consistently observed. From tissue showing systemic symptoms on such Black Turtle Soup plants SBMV-B and D were isolated. These two strains never again produce necrotic local lesions when inoculated to Black Turtle Soup plants. The systemic symptoms in Black Turtle Soup plants inoculated with SBMV-A or C were slow to appear, generally between the second and third week after inoculation. In contrast, systemic symptoms appeared 1 wk after inoculation when SBMV-B or D were used. When a mixture of A and B or C and D was inoculated to Black Turtle Soup plants, necrotic local lesions appeared in 5 days and systemic symptoms in 1 wk.

The symptoms induced by SBMV-C in most bean cultivars were similar to those induced by SvBMV (4,12) but there were significant differences in the remainder of the host range.

The potential for variability of SBMV-A is great. Mutation occurred rapidly under greenhouse conditions. The resistance to SBMV-A and C by the cultivar Black Turtle Soup was readily overcome. In contrast, the other strains of SBMV are apparently very stable. Lamptey and Hamilton (5) speculate on the evolutionary trend in SBMV strains, indicating that various types of SBMV may have arisen by mutation from SBMV-GH. They suggest that continuous propagation of this strain in a single cultivar of bean or cowpea under conditions of high temperature could result in mutant strains. The current studies also indicate that instability may be an inherent property of certain strains.

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