Some Properties of an Isolate of the Soybean Stunt Strain of Cucumber Mosaic Virus

K. Hanada and H. Tochihara

Plant pathologists, Institute for Plant Virus Research, Tsukuba Science City, Ibaraki, 305, Japan.
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ABSTRACT

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An isolate of soybean stunt virus (SSV-A) obtained from mottled soybean seeds was characterized and compared with isolates of cucumber mosaic virus (CMV). SSV-A had a similar host range to that of several CMV strains, although some differences were detected. In agar gel diffusion tests, spur formation was observed between purified SSV-A and two serologically different CMVs. Polyacrylamide gel electrophoresis (PAGE) showed that SSV-A contained five major RNA species (RNAs) that had almost the same molecular weights of CMV-RNAs. A mixture of the three largest RNAs of SSV-A had the highest infectivity. SSV-A had one kind of protein with a molecular weight of ~24,000, which comigrated with CMV-protein in PAGE. Pseudorecombinants were constructed between SSV-A and CMV by exchange of RNA 3. It was concluded that SSV-A is a soybean strain of CMV with a unique serotype.

An aphidborne virus differing from soybean mosaic virus was isolated from soybean plants in Japan in 1958 and named soybean stunt virus (SSV) (9). SSV induced mild mosaic and stunting on infected soybean plants, which later produced mottled seeds with concentric rings. SSV was transmissible through soybean seeds at a rate of 40–100% (9,14). Virions of SSV were reported to be spherical particles 25–28 nm in diameter, serologically closely related to cucumber mosaic virus (CMV) (14). SSV was also isolated in Indonesia in 1975 (12).

In this paper, we describe some host range properties, serological specificity, five major RNAs, a tripartite genome, and coat protein of an isolate of SSV. We also report pseudorecombination between SSV and CMV by exchange of RNA 3.

MATERIALS AND METHODS

Virus, virus purification, and serology. SSV-A was isolated from mottled seeds of soybean, *Glycine max,* and was characterized after single lesion isolation using *Chenopodium quinoa* as a local lesion host. Three CMV isolates (CMV-Y, -P and -E), peanut stunt virus (PSV) and chrysanthemum mild mottle virus (CMMV) were described before (6). All viruses were purified from inoculated tobacco, *Nicotiana tabacum* 'Ky 57' or 'Xanthi nc,' or *N. clevelandii* leaves as described by Tochihara (16). Antisera against CMV-Y, CMV-P, and CMMV were prepared previously, and agar gel diffusion tests were done in 0.7% agarose as described previously (6,16).

Electrophoresis of viral RNAs. Polyacrylamide gel electrophoresis (PAGE) of RNA was done by similar methods described by Takanami and Kubo (15). To a purified virus solution 1% sodium dodecyl sulfate (SDS) and 10% RNase-free sucrose were added. After 1 min of incubation at 40 C, SDS-disrupted virus samples were loaded on 2.5% gels and run at 5 mA per gel for 2–3 hr at room temperature. Gel scanning, RNA recovery from gels, and inoculation of RNAs were done as described previously (6). In all trials except one, ethanol-precipitated RNAs were used directly for bioassay and exchange of RNA 3. In one experiment, recovered RNAs were rerun by PAGE to reduce contaminated RNAs. Molecular weight (MW) markers used were tobacco mosaic virus-RNA, MW 2.0×10^6 (1), and *Escherichia coli* 23S and 16S ribosomal RNA obtained from Miles Laboratories, MW 1.07 and 0.55×10^6, respectively (13). The MWs of viral RNAs were determined by plotting the logarithms of the MWs of marker RNAs against migration distances.

Electrophoresis of viral proteins. PAGE of proteins was done by the method of Laemmli (10) by using slab gels (12×18 cm, 1.5 mm thick) of 3% acrylamide for stacking (3 cm long) and 10% for separation (9 cm long). One volume of 0.13 M tris (hydroxymethyl)aminomethane HCl buffer, pH 6.8, 2% SDS, 1% sucrose, and 5% mercaptoethanol were added to purified virus solution, which was subsequently boiled for 2 min. Samples were run at 30 mA per gel for 2 hr, and gels were stained with Coomassie brilliant blue. MW markers used were bovine serum albumin (MW 68,000), ovalbumin (45,000), and chymotripsinogen A (25,000), all purchased from Boehringer Mannheim, Mannheim, West Germany. Mobilities were determined relative to that of cytochrome c used as an internal marker.

Exchange of RNA 3 between SSV and CMV. RNA 1+2 preparations of CMV-E and SSV-A were mixed with RNA 3 of SSV-A and CMV-E, respectively, then inoculated to *C. quinoa* plants. For the control experiment, homologous mixtures of RNA 1+2 and RNA 3 from either virus were inoculated at the same time. Several single lesions were isolated from inoculated leaves for all combinations of RNAs. Then, the serotype of each single isolate was determined by gel diffusion tests, and symptoms on asparagus bean, *Vigna ungiculata* subsp. *sesquipedalis* 'Kurodane,' were observed after subculturing.

RESULTS

Comparison of host range between SSV-A and CMV strains. SSV-A and CMV strains (CMV-Y, -P, and -E) caused similar reactions on *N. glutinosa,* tobacco cultivar 'Ky 57,' asparagus bean 'Kurodane,' and cucumber (*Cucumis sativus* 'Chicago Pickling') plants. Differential indicator plants were soybean cultivars Toyosuzu or Tsurunoko and tomato (*Lycopersicon esculentum* 'Fukuju No. 2'). Plants of cultivars Toyosuzu and Tsurunoko infected with SSV-A produced mosaic, whereas CMV strains induced no symptoms and were not recovered from inoculated leaves of soybean. Tomato infected with any CMV showed mosaic, while SSV-A caused no obvious symptoms on tomato. Typical host reactions are shown in Table 1.

Serological specificity of SSV-A. Purified SSV-A reacted with antisera against CMV-Y and CMV-P in agar gel diffusion tests by forming spurs with purified CMV-Y and CMV-P (Fig. 1). Spur formation was observed consistently with six different purified virus samples. No reactions were detected between purified SSV-A and CMMV-antiserum in gel diffusion tests. CMV-E and CMV-Y

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were indistinguishable by these three antisera. No reactions were observed between purified PSV or CMMV and antisera against the CMV strains under the conditions that were used.

Electrophoresis of viral RNAs and protein. Electrophoretic patterns of SDS-disrupted SSV-A and CMV-Y are shown in Fig. 2. Both viruses had similar patterns and contained five major RNAs. MWs of RNA 1-4 of SSV-A under nondenaturing conditions were calculated to be 1.22, 1.13, 0.83, and 0.42×10^6, respectively. MWs of RNA 1-4 of CMV-Y under similar conditions were found to be 1.25, 1.14, 0.87, and 0.42×10^6, respectively. These values are means of six determinations using two different single lesion isolates of each virus. MW of RNA 5 of both viruses was about 0.10×10^6.

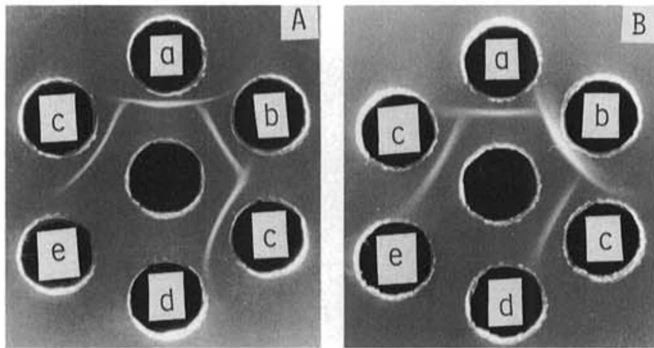

Fig. 1. Agar gel diffusion tests of SSV-A, CMV-Y, CMV-P, PSV, and CMMV. Each outer well contains 100 μg of purified virus: **a,** CMV-Y; **b,** CMV-P; **c,** SSV-A; **d,** CMMV and **e,** PSV. Central wells contain antisera to **A,** CMV-Y and **B,** CMV-P.

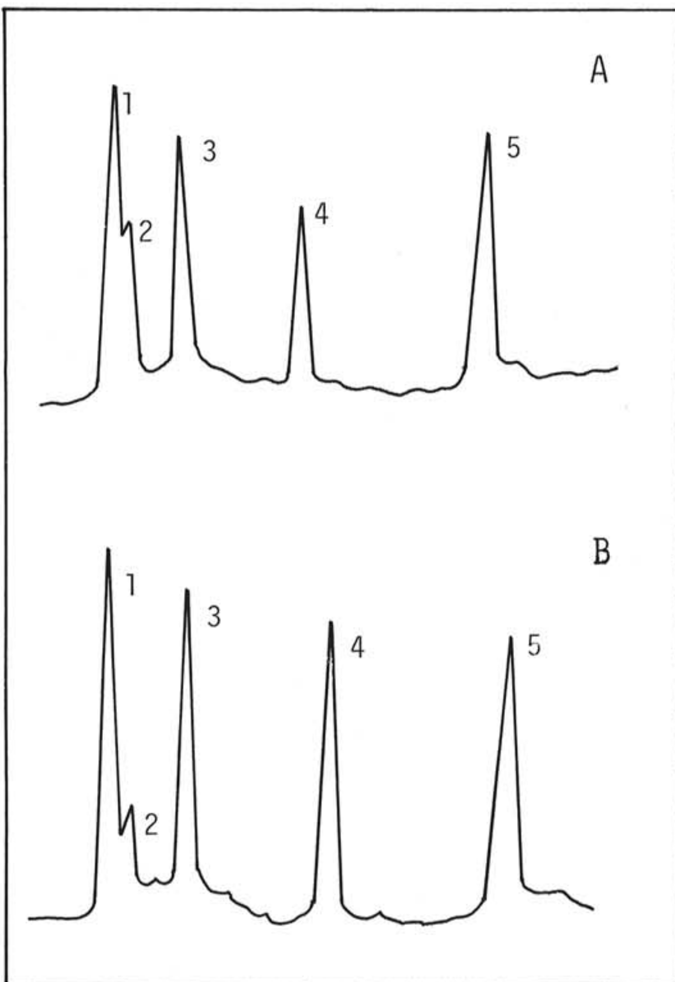

Fig. 2. Electrophoretic patterns of RNAs of SSV-A and CMV-Y. SDS disrupted virus (about 70μg virus per gel) was subjected to PAGE in 2.5% gels. Migration is from left to right. **A,** SSV-A and **B,** CMV-Y.

Coat protein of SSV-A migrated as a single component and had the same mobility of coat proteins of CMV-Y and -P (Fig. 3). MWs of coat proteins of these viruses were calculated to be about 24,000. CMV-E had five major RNAs and one kind of protein with MWs similar to those of CMV-Y.

Infectivity of viral RNAs. Infectivity of the three largest RNAs of SSV-A and CMV-E was tested by bioassay after one or two cycles of PAGE separation of RNAs. As shown in Table 2 (Experiments 1 to 3), two of the three RNAs had no or very little infectivity, and the addition of the remaining RNAs to two RNAs induced or enhanced infectivity at least 10 to 40 times. Residual infectivity of RNA 1+2 of SSV-A was lost after two cycles of PAGE separation, but RNA 1+2 of CMV-E still had residual infectivity, probably due to contaminated RNA 3 in RNA 1+2 (Experiment 4 in Table 2).

Pseudorecombination between SSV-A and CMV-E. Single lesion isolates (SLIs) were obtained after exchange of RNA 3 between SSV-A and CMV-E. Five out of seven SLIs had the serotype of RNA 3 donor (Experiment 1 in Table 3) when slightly contaminated RNA samples were used for exchange. When less contaminated samples were used, all except one SLI had the serotype of RNA 3 donor (Experiment 2 in Table 3). SSV-A produced necrotic local lesions in inoculated leaves of 'Kurodane,' while CMV-E induced chlorotic spots. All SLIs obtained in RNA 3 exchange produced symptoms on plants of cultivar Kurodane similar to symptoms induced by RNA 1+2 donor. All SLIs obtained by parental combinations of RNA 1+2 and RNA 3

TABLE 1. Typical host reactions induced by SSV-A and CMVs

Virus	*N. glutinosa*	Tobacco Ky 57	Tomato Fukuju No. 2	Soybean Toyosuzu	Cucumber Chicago Pickling
SSV-A	M[a]	(mM)	–	M	M
CMV-Y	YM	YM	M	–	M
CMV-P	M	(mM)	mM	–	M
CMV-E	M	M	M	–	M

[a] Systemic symptoms occurred: M = mosaic, YM = yellow mosaic, mM = mild mosaic, – = no symptoms observed. Letters in parentheses indicate that symptoms did not always appear.

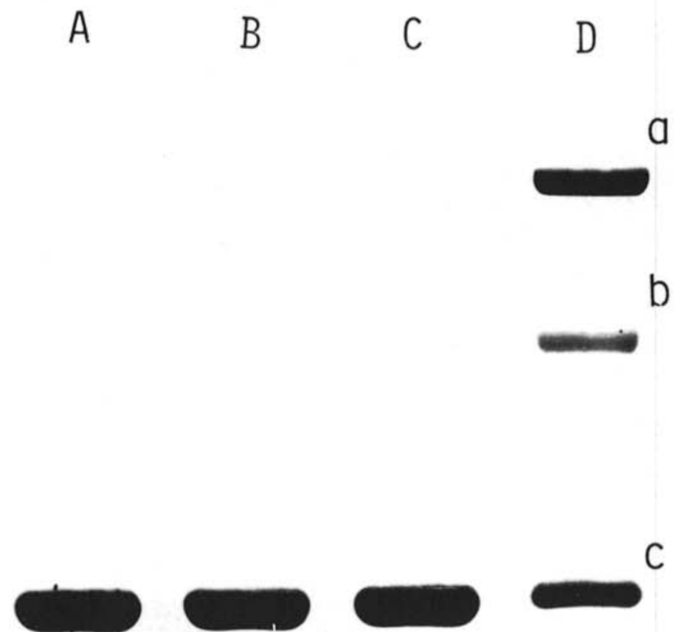

Fig. 3. Electrophoretic patterns of coat proteins of SSV-A, CMV-Y and CMV-P. Purified virus (about 40 μg per pocket of a slab gel) was subjected to PAGE after SDS disruption as described in materials and methods. **A,** SSV-A, **B,** CMV-P, **C,** CMV-Y, and **D,** marker proteins: **a,** bovine serum albumin (MW 68,000), **b,** ovalbumin (45,000), and **c,** chymotripsinogen A (25,000).

retained serotype and symptoms on Kurodane of parental virus. Our results showed: that exceptional SLIs with the serotype of RNA 1+2 donor were possibly generated by contaminated RNA 3 in the original RNA 1+2 samples of CMV-E; that RNA 3 determined the serotypes of SSV-A and CMV-E; and that RNA 1 or RNA 2 or both decided symptom types on Kurodane of these viruses. Gel diffusion tests of pseudorecombinants (PRs) and parental viruses are shown in Fig. 4. Reexchange of RNA 3 between two types of PRs produced parental viruses, considering serotype as a marker on RNA 3 and symptoms on Kurodane as a marker of RNA 1+2, although one exceptional isolate was obtained which probably originated from contaminated RNA (Table 4).

DISCUSSION

Takahashi et al (14) reported on a host range of SSV using 111 plant species and their results compared to CMV are as follows: none of SSV isolates induced symptoms on tomato, tobacco, and cucumber plants which produced clear symptoms after CMV infection; SSV infection caused mosaic symptoms on soybean, which produced no symptoms after CMV infection; and both viruses produced similar symptoms on cowpea and *N. glutinosa* (14). Using different cultivars of tomato and soybean we confirmed these reactions (Table 1), but we found that SSV-A produced mosaic on cucumber Chicago Pickling and occasionally induced mild mosaic on tobacco Ky 57.

French workers have assigned a number of CMV isolates to two serotypes (2). We reported that CMV strains isolated in Japan could be divided into the Y and P serotypes (6,17). Agar gel diffusion tests in Fig. 1 demonstrated that SSV-A belonged to a different serotype. The relationships between the French and Japanese serotypes of CMV are uncertain.

Cucumber mosaic virus contains four or five major RNAs (8,11) and one kind of coat protein (4,7). We recently showed that CMV-Y, -P and -E contained four or five major RNAs by PAGE (6), and as shown in Fig. 2, SSV-A contained five major RNAs like CMV-Y. Apparent MWs of RNAs of SSV-A were determined, which were quite close to values of comparable RNAs of CMV-Y. These values of MWs of RNAs were similar to previously reported values under similar conditions (4,6,11). MWs of coat proteins of SSV-A and three CMV strains were almost identical and are very similar to values reported by other workers (4,7). Infectivity assay of SSV-A showed that SSV-A had a tripartite genome like CMV-E (Table 2) and other CMVs (6,11). In 1971, SSV was first reported to be an isolate of CMV (18), then they were considered to be different (14). We found that SSV-A and CMV were biochemically similar and

TABLE 2. Infectivity of RNAs of SSV-A and CMV-E

RNAs in inoculum	Infectivity[a]					
	Exp. 1	Exp. 2	Exp. 3		Exp. 4	
	SSV-A	CMV-E	SSV-A	CMV-E	SSV-A	CMV-E
1 + 2	5	6	1	5	0	2
1 + 3	3	5	0	2	NT	NT
2 + 3	1	1	2	0	NT	NT
3	NT	NT	NT	NT	0	0
1 + 2 + 3	177	65	40	143	12	63

[a]Total numbers of local lesions in four half-leaves of *Chenopodium quinoa*. NT = not tested. SDS disrupted viruses (1 mg virus sample was loaded onto three gels) were subjected to PAGE at 5 mA per gel for 2–3 hr, then each RNA was recovered from gels by phenol extraction and ethanol precipitation. RNAs used in Experiments 1 to 3 were obtained by one cycle of PAGE separation and RNAs in Experiment 4 were obtained by rerun of PAGE-separated-RNA 1+2 or RNA 3 in different gels under similar conditions. Design of bioassay was done as described by Gibbs and Harrison (3). Precipitated RNAs were inoculated at the same dilution.

TABLE 3. Serotypes of and symptoms caused on asparagus bean cultivar Kurodane by single-lesion isolates obtained by exchange of RNA 3 between SSV-A and CMV-E

RNA donor[a]		Number of single lesion isolates with:			
		Serotype of:		Symptoms on Kurodane similar to:	
RNA 1+2	RNA 3	CMV-E	SSV-A	CMV-E	SSV-A
Expt. 1					
CMV-E	CMV-E	3	0	3	0
SSV-A	SSV-A	0	3	0	3
CMV-E	SSV-A	2	2	4	0
SSV-A	CMV-E	3	0	0	3
Expt. 2					
CMV-E	CMV-E	8	0	8	0
SSV-A	SSV-A	0	4	0	4
CMV-E	SSV-A	1	7	8	0
SSV-A	CMV-E	6	0	0	6

[a]Infectivity of RNAs in inoculum was tested prior to use in order to check purity of RNAs and results are shown in Table 2. RNAs used in Experiment 1 are from Experiment 1 and 2 in Table 2, and Experiment 2 are from Experiment 4 in Table 2, respectively.

TABLE 4. Regeneration of parent viruses by RNA 3 exchange between two types of pseudorecombinants (PRs)

RNA donor[a]		Number of single-lesion isolates with:			
		Serotype of:		Symptoms on Kurodane similar to:	
RNA 1+2	RNA 3	CMV-E	SSV-A	CMV-E	SSV-A
C S[a]	C S	0	4	3	0
S C	S C	4	0	0	3
C S	S C	5	0	5	0
S C	C S	1	6	0	7

[a]RNAs in inoculum were obtained from PRs constructed in Experiment 1 in Table 3. C = CMV-E and S = SSV-A. The first and the second letters represent the origin of RNA 1+2 and RNA 3, respectively. Purified virus samples of PRs were subjected to one cycle of PAGE and recovered RNAs were inoculated to *Chenopodium quinoa* in combinations. After lesion isolation, serotype and symptoms of each single-lesion isolate were determined.

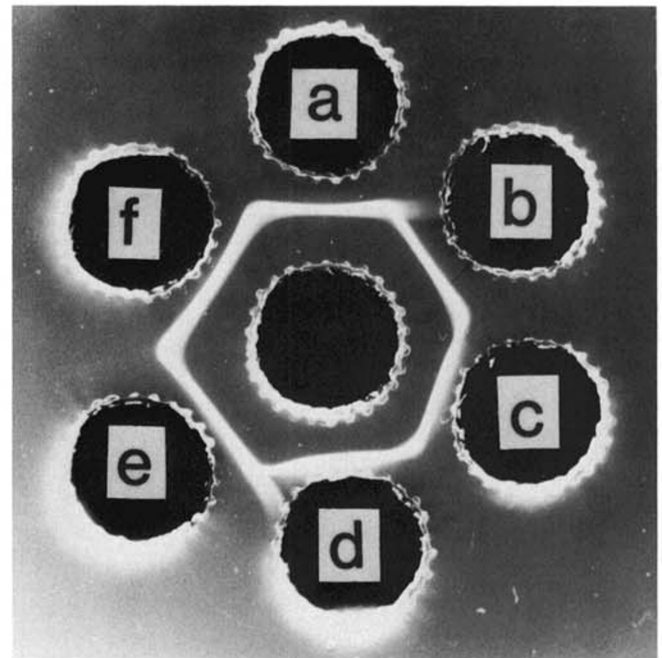

Fig. 4. An agar gel diffusion test of pseudorecombinants (PRs) constructed by RNA 3 exchange between SSV-A and CMV-E. Each outer well contains 200 μg purified virus: **a,** CMV-E, **b,** a virus made from RNA 1+2 and RNA 3 of SSV-A, **c,** a PR containing RNA 1+2 of CMV-E and RNA 3 of SSV-A, **d,** SSV-A, **e,** another type of PR containing RNA 1+2 of SSV-A and RNA 3 of CMV-E, and **f,** a virus made from RNA 1+2 and RNA 3 of CMV-E. The central well contains antiserum to CMV-Y.

conclude that SSV-A is a soybean strain of CMV with a unique serotype.

Pseudorecombination experiments (Table 3) confirmed a close relatedness between SSV and CMV, since no PRs have been obtained between unrelated viruses and only one type of PRs obtained between distantly related viruses (3,5,6). We located determinants of symptoms on asparagus bean on RNA 1+2 and of serotype on RNA 3 by analysis of PRs from SSV-A and CMV-E. Two studies with PRs of cucumoviruses showed similar results (5,6). It was also reported that the smallest RNA of CMV (CARNA5) is associated with necrosis on tomato (8). CMV-Y induced necrosis on tomato, while SSV-A, CMV-E, and -P did not. SSV-A may be useful to locate symptom and seed transmissibility determinants by analysis of PRs.

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