III. Virus diseases of legumes

SOYBEAN MOSAIC VIRUS ISOLATED FROM SOYBEANS IN THAILAND[1)]

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Abstract

Four isolates of soybean mosaic virus (SMV) from soybeans in Thailand were identified by host range, serology, transmission and electron microscopy. The host range of the four isolates differed in reactions in *Chenopodium amaranticolor, C. quinoa, Tetragonia expansa* and *Phaseolus vulgaris* 'Top Crop'. Isolates of SMV from Thailand could be classified into two serological groups which were different from a SMV isolate from Japan. The serological relationship did not appear to be correlated with the host reactions.

1. Introduction

Soybean mosaic virus (SMV) is considered to be the world's most prevalent virus in soybean, mainly because the virus is seed-borne and can be easily transmitted by many aphid species in a non-persistent manner (1). The virus is also mechanically transmitted. Deema (1977) (3) reported that SMV and soybean yellow mottle virus occurred naturally in soybeans in Thailand. However, these viruses have not been studied in detail and very little is known about the properties of these viruses.

This paper reports the results of an investigation carried out to characterize the SMV isolated from soybeans in Thailand.

2. Materials and methods

We used four isolates, SMV-12B, SMV-27, SMV-43 and SMV-124, obtained from naturally infected, field-grown soybeans in various growing areas in Thailand in 1979. Various potyviruses and their antisera, including a Japanese SMV isolate (SMV-H) from Hokkaido, were used to compare serological relationships. The virus isolates and antisera used are listed in Table 1.

Sap inoculation was made by rubbing the carborundum-dusted leaves of test plants with a piece of cotton soaked in a homogenate of infected leaves prepared in 0.1 M phosphate buffer, pH 7.0. Aphid transmission of the viruses was tested by allowing an acquisition access period of 3 to 10 min with 1-2 hr of preacquisition starvation,

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Table 1. Sources of virus isolates and antisera to virus used in the comparison studies

Virus isolate and antiserum to virus	Locality	Source
SMV-H[a)]	Hokkaido, Japan	T. Tsuchizaki *et al.*
BCMV[b)]	Hokkaido, Japan	T. Tsuchizaki *et al.*
AzMV[c)]	Hokkaido, Japan	K. Yoshida
PVY[d)]	Hokkaido, japan	T. Tsuchizaki
antiserum to SMV-H		T. Tsuchizaki
antiserum to BCMV		T. Tsuchizaki
antiserum to BYMV-P[e)]		T. Tsuchizaki
antiserum to PVY		Y. Saito
antiserum to BMV[f)]		I. Fujisawa
antiserum to AzMV		N. Iizuka

a) SMV-H: an isolate of soybean mosaic virus from Hokkaido
b) BCMV: an isolate of bean common mosaic virus
c) AzMV: an isolate of azuki bean mosaic virus
d) PVY: an isolate of potato virus Y
e) BYMV-P: P strain of bean yellow mosaic virus from Hokkaido
f) BMV: isolate of beet mosaic virus

followed by an overnight inoculation access period on healthy test plants. Five to ten aphids were placed on each of the healthy plants.

Purified virus was prepared from infected leaves of soybean plants. Leaf tissues were homogenized in 0.5 M citrate buffer (pH 7.2) containing 1% 2-mercapto-ethanol (3 ml/g tissue). After addition of carbon tetrachloride to the extract to reach 25% (v/v), the mixture was shaken for 15 min and the emulsion was broken by centrifugation at 8,000 g for 10 min and the aqueous phase was recovered. Polyethylene glycol 6,000 (PEG)and triton X-100 were added to the aqueous phase to give a final concentration of 5 and 1%, respectively. After stirring for 1-2 hr, the mixture was centrifuged at 8,000 g for 15 min, and the pellets were resuspended in 0.5 M K-phosphate buffer (pH 7.5) containing 0.01 M magnesium chloride, and clarified by centrifugation at 5,000 g for 10 min. The PEG purification and clarification procedures were repeated twice, then the preparation was centrifuged at 120,000 g for 60 min. The pellets were resuspended in the above phosphate buffer, and clarified by low speed centrifugation as described above. The resulting supernatant fluid was centrifuged at 60,000 g for 3 hr in 10-40% linear sucrose density gradient columns prepared in 0.5 M K-phosphate buffer (pH 7.5) containing 0.01 M magnesium chloride. After centrifugation, the gradient columns were scanned at 254 nm and fractionated with an ISCO Model 640 density-gradient fractionator. Ultraviolet-absorbing fractions were pooled and centrifuged at 120,000 g for 60 min. The virus pellets were resuspended in 0.005 M K-phosphate buffer (pH 7.0).

For electron microscopic observation, small pieces of infected soybean leaves were fixed in 3% glutaraldehyde in 0.1M phosphate buffer (pH 7.0) for 4 hr at 4°C and post-fixed in 1% osmium tetroxide in the same buffer for 2 hr at 4°C. After dehydration with an acetone series, they were embedded in epoxy resin. The sections were stained

with uranyl acetate and lead citrate.

For the study of the physical properties of these viruses, leaves of soybean with systemic infection were macerated in a mortar with a pestle. The crude sap was expressed through cheesecloth pads, and a 1 : 10 dilution was made for each isolate with 0.1 M phosphate buffer, pH 7.0. The diluted sap was divided into three parts for the study of the thermal inactivation point, dilution end-point, and longevity of the virus in vitro.

Antiserum against the virus was produced in a rabbit by three intramuscular injections of purified virus emulsified with Freund's complete adjuvant (1: 1, v/v) at 3 week intervals. Antiserum was collected from the rabbit 4 weeks after the final injection. Ouchterlony double-diffusion tests were conducted in 0.75% agar with 0.85% sodium chloride, 0.5% lithium 3,5-diiodosalicylate, and 0.05% sodium azide.

3. Results

1) Host range

Reactions of the tested plants to infection by the four isolates of SMV are summarized in Table 2. *Glycine max* and *Phaseolus vulgaris* 'Tsurunashi Kintoki' were susceptible to all of the four SMV isolates and showed systemic symptoms. Local lesions developed on inoculated leaves of *Tetragonia expansa, Chenopodium amaranticolor* and *C. quinoa* after inoculation with SMV-27, and on *P. vulgaris* 'Top Crop' inoculated with SMV-12B (Table 2).

2) Virus transmission

Aphis glycines, Myzus persicae, and *Aulacorthum solani* were able to transmit SMV in a non-persistent manner (Table 3). All the seedlings grown from seeds collected from three cultivars of soybean plants inoculated with SMV-27 or SMV-43 in the greenhouse were healthy (Table 4). However, one out of 179 seedlings grown from Okuharawase soybean plants infected with SMV-12B in the greenhouse was infected with the virus.

3) Particle morphology

Electron microscopic observation of purified viruses or leaf-dip preparations from infected soybean leaves stained with 1% phosphotungstic acid revealed that the particles of the four SMV isolates were flexuous rods about 750 nm in length.

4) Inclusion bodies

All four SMV isolates used in this study induced pinwheel and circular inclusion bodies in various areas of the cytoplasm of infected soybean cells.

5) Serological test

Antiserum against SMV-27 or SMV-12B had a dilution end point of 1: 512 in ring interface precipitin test. Serological relationships among SMV isolates were tested in Ouchterlony double-diffusion test using SMV-12B, SMV-27 and SMV-H antisera

Table 2. Host reactions to infection with the four isolates of soybean mosaic virus from Thailand

Plant species	Soybean mosaic virus isolates[a]			
	SMV-12B	SMV-27	SMV-43	SMV-124
Chenopodium amaranticolor	—	L	—	—
C. quinoa	—	L	—	LL
Spinacia oleracea	—	LL	—	LL
Gomphrena globosa	LL			LL
Tetragonia expansa	—	L	—	LL
Brassica rapa	—	—	—	—
Arachis hypogaea	LL			—
Astragalus sinicus	LL			
Glycine max 'Shirotsurunoko'	CS,M	L,CS,M	CS,M	CS,M
'Okuharawase'	CS,M	CS	L,CS	CS,M
'Isuzu'		L,M	CS	
Lathyrus odoratus	—			
Lupinas luteus	LL			
Medicago sativa	—			
Phaseolus angularis	LL	M	LS	LS
P. vulgaris 'Tsurunashi Kintoki'	M	L,CS	L,CS,M	M
'Top Crop'	L	—	—	—
'Yamashiro Kurosando'	—			LL
Pisum sativum	LL	—	—	LL
Trifolium pratense	—	—	—	—
T. repens	—	—	—	—
Vicia faba	LL	—	LL	—
Vigna mungo	—	—	—	—
V. radiata	LL	—	—	LL
V. sesquipedalis	—	—		—
V. sinensis	—	LS	—	LS
Datura stramonium	—			LL
Lycopersicon esculentum	—			—
Nicotiana clevelandii	LS			LS
N. glutinosa	—	—	—	—
N. tabacum 'Bright Yellow'	—	—	—	—
Petunia hybrida	—	—	—	LL
Sesamum indicum	LS	—	—	—
Cucumis sativus	—	—		
Zinnia elegans	—	—	—	—

a) L: necrotic or chlorotic local lesions on inoculated leaves, M: mosaic on non inoculated leaves, CS: chlorotic spot on non inoculated leaves, LL: symptomless local infection, LS: symptomless systemic infection, —: no infection.

Table 3. Transmission of four isolates of soybean mosaic virus from Thailand by aphids

Aphids	Soybean mosaic virus isolates[a]			
	SMV-12B	SMV-27	SMV-43	SMV-124
Aphis glycines	8/10	9/10	—	—
Myzus persicae	—	—	6/8	3/4
Aulacorthum solani	—	—	4/4	2/4

a) Number of infected plants/number of test plants, —: not tested.

Table 4. Seed transmission of three isolates of soybean mosaic virus from Thailand

Soybean variety	Soybean mosaic virus isolates[a)]		
	SMV-12B	SMV-27	SMV-43
Shirotsurunoko	—	0/234	0/224
Isuzu	—	0/38	0/127
Okuharawase	1/179	0/76	0/90

a) Number of infected seedlings/number of seedlings tested, —: not tests.

Table 5. Serological reaction among isolates of soybean mosaic virus in agar gel diffusion plates containing 0.5% lithium 3,5-diiodosalicylate

Antisera	Virus isolates			
	SMV-H	SMV-27	SMV-12B	SMV-124
SMV-H	+	+*	+*	+*
SMV-27	+*	+	+*	+
SMV-12B	+*	+*	+	+*

* Spur reaction was observed with the virus isolate homologous to the antiserum used.

Table 6. Serological reaction between the SMV-27 isolate of soybean mosaic virus and several other viruses of the potato virus Y group in agar gel diffusion plates containing 0.5% lithium 3,5-diiodosalicylate

Virus isolates	Antisera						
	SMV-27	BYMV-P	AzMV	PVY	TuMV	BMV	BCMV
SMV-27	+	-	+*	-	-	-	+*
AzMV	+*	0	+	0	0	0	0
PVY	-	0	0	+	0	0	0
BCMV	+*	0	0	0	0	0	+

* Spur reaction was observed with the virus isolate homologous to the antiserum used, 0: not tested.

(Table 5). With antiserum to SMV-27, SMV-27 spurred over SMV-12B and SMV-H, but not over SMV-124. SMV-124 formed lines of identity with SMV-27. With antiserum to SMV-12B, SMV-12B spurred over SMV-27, SMV-124 and SMV-H (Fig. 4A). With antiserum to SMV-H, SMV-H spurred over SMV-27, SMV-124 and SMV-12B.

When antisera to six other potyviruses were used, SMV-27 did not react with P strain of bean yellow mosaic virus (BYMV-P), potato virus Y (PVY), turnip mosaic virus (TuMV) or beet mosaic virus (BMV), but reacted with azuki bean mosaic virus (AzMV) and bean common mosaic virus (BCMV) (Table 6). A spur was observed between the precipitin line of SMV-27 and AzMV or BCMV. Antiserum against SMV-27 did not react with PVY, but reacted with AzMV and BCMV, spur was observed between the precipitin lines of SMV-27 and AzMV or BCMV.

6) Stability in sap

The thermal inactivation point (10 min) of SMV-12B, SMV-27 or SMV-124 was 50–60°C. The dilution end-point of SMV-12B or SMV-27 was 10^{-3}–10^{-4}, but that of SMV-124 was at least 10^{-6}. In crude sap both SMV-12B and SMV-27 had a longevity in vitro of 1–4 days at room temperature but that of SMV-124 was 14–21 days.

4. Discussion

Results from our studies on particle size and morphology, host range and symptomatology, transmission and the serological relationship of the four virus isolates from Thailand clearly indicated that these viruses are SMV. SMV occurs in most countries where soybeans are grown. The wide distribution of SMV could be attributed to its being seed-borne and having a comparatively wide range species of aphid vectors (1). Two SMV isolates used in our experiments (SMV-27 and SMV-43) were not transmissible through soybean seed and the frequency of seed transmission of one SMV isolate (SMV-12B) was very low. It was reported that the rate of seed transmission of SMV depended on cultivars of soybeans and virus strains (6). The SMV isolates collected from Thailand may be transmissible through seeds of other cultivars of soybean.

It has been reported that different isolates of SMV show a considerable variability in host range (2, 4, 5, 12). Takahashi *et al.* (1980) (6) reported five strains of the virus on the basis of symptoms and ability to infect different soybean cultivars. In this study, the four SMV isolates collected from Thailand also showed differences in host reactions. The results from our Ouchterlony double-diffusion tests also indicated that SMV-12B, SMV-27 and SMV-H (an isolate of SMV from Japan) are serologically related but not identical, and SMV-27 and SMV-124 are serologically undistinguishable. Very little information on the serological relationship among various strains in different geographical areas is available. SMV isolates from various locations in Thailand could be classified into two serotypes which are serologically different from a SMV isolate from Japan. Although members of these two serotypes had many properties in common, they induced different symptoms in infected *C. amaranticolor, C. quinoa, T. expansa,* and 'Top Crop' bean. Apparently, the serological relationships and host reactions did not seem to be correlated.

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