Comparisons among Indonesian Isolated and Japanese Strains of Soybean Stunt Virus

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Introduction

One of the limiting factors hampering soybean production is virus disease. Recently, many kinds of soybean viruses have been identified in Indonesia[8]. Soybean stunt virus (SSV) was first reported in Japan[4] and then Indonesia[5]. Soybean plants infected with SSV generally show mosaic and slightly stunting symptoms, and produce many brown mottled seeds. The virus is easily transmitted by aphids and mechanical inoculations, and also through seeds in a high rate of transmission. The virus reduces soybean production up to 50%. In Japan there are five strains of SSV which were classified into SSV-A, -AE, -B, -C and -D on the basis of symptoms and pathogenicity to soybean varieties[3,6]. These strains are useful for study of epidemiology, distribution and screening tests for resistance of SSV.

In this paper we present the result of host range, reactions to soybean varieties, purification, serological relationships and seed transmission of Indonesian isolates and Japanese strains of SSV.

Host range and symptomatology

Two SSV isolates, SSV-4-2A and SSV-13C, were isolated from naturally infected soybean plants varied in symptoms in Indonesia. Two Japanese strains, SSV-AE and SSV-B, were used for comparison.

Host range of the viruses was determined by mechanical inoculations of 24 plant species belonging to seven families. SSV-4-2A, -13C, -AE and -B caused the similar reactions in 13 plant species belonging to six families, but peanut (*Arachis hypogaea*), *Nicotiana sylvestris*, *Petunia hybrida* and *Physalis floridana* were not infected with them (Table 1).

Differential indicator plants were soybean varieties and six other plant species belonging to Leguminosae and Compositae (Table 2). Thirty eight varieties of soybean were classified into 10 groups on the basis of the symptoms (Table 2). Only Hakuho No. 1, Kariutakiya No. 28 and Dewamusume were infected with SSV-B, SSV-AE and SSV-4-2A, respectively. Shiratama No. 10 infected with SSV-4-2A or SSV-B showed mosaic symptoms, whereas SSV-13C or SSV-AE caused no infection.

Reactions of tested French bean (*Phaseolus vulgaris*), pea (*Pisum sativum*), broad bean (*Vicia faba*), asparagus bean (*Vigna unguiculata* subsp. *sesquipedalis*), cowpea (*V. unguiculata* subsp. *unguiculata*) and *Zinnia elegans* made it possible to differentiate SSV-4-2A from SSV-13C, while SSV-13C and SSV-AE were indistinguishable each other (Table 2). Inoculated leaves of asparagus bean infected with SSV-4-2A produced large chlorotic and necrotic spots, while SSV-13C and SSV-AE caused small necrotic spots and SSV-B caused irregular chlorotic spots (Plate 1).

Table 1. Host reactions to Indonesian isolates (4-2A and 13C) and Japanese strains (AE and B) of soybean stunt virus

Family	Species	Symptoms	
		IL	NIL
Aizoaceae	*Tetragonia expansa*	cs	—
Amaranthaceae	*Gomphrena globosa*	o	lat
Chenopodiaceae	*Chenopodium quinoa*	cs	—
	C. amaranticolor	cs	—
Cucurbitaceae	*Cucumis sativus*	lat	—
Leguminosae	*Arachis hypogaea*	—	—
	Vigna radiata cv. Meiryokuzu	ns	—
Solanaceae	*Datura stramonium*	cs	—
	Nicotiana benthamiana	o	lat
	N. clevelandii	o	lat
	N. glutinosa	o	(m)
	N. megalosiphon	o	lat
	N. rustica	o	lat
	N. sylvestris	o	—
	N. tabacum cv. Xanthi nc	o	lat
	Petunia hybrida	o	—
	Physalis floridana	o	—

IL: Inoculated leaves, NIL: Non-inoculated leaves.
cs: chlorotic spot, lat: latent infection, m: mild mottle, ns: necrotic spot,
o: no symptoms appeared, —: no infection, ( ): sometimes symptoms appeared.

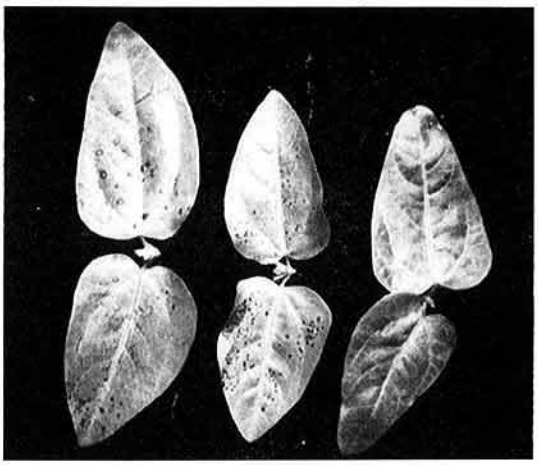

Plate 1. Inoculated leaves of asparagus bean (Kurodane sanjaku) produced large chlorotic and necrotic spots by SSV-4-2A (left), small necrotic spots by SSV-13C (center), and irregular chlorotic spots by SSV-B (right)

Only SSV-B caused systemic symptoms on French bean, asparagus bean and copwea.

As the result of Table 2, SSV-4-2A, -13C, -AE and -B were distinguished on the basis of differences in pathogenicity and virulence towards the tested plant species and varieties.

Purification of SSV-4-2A

Inoculated leaves of *N. tabacum* cv. Xanthi nc were collected seven days after inoculation with SSV-4-2A. Purification was carried out by the same procedure for cucumber mosaic virus (CMV)[7]. The purified virus preparations had an ultraviolet light absorption spectrum characterized of that of nucleoprotein with an A_{260}/A_{280} value of 1.76. The purified virus particles were isometric in shape and about 28 nm in diameter (Plate 2). Assuming the extinction coefficient ($E^{0.1\%}_{10mm}$) at 260 nm was 5.0 as for the purified particles of cucumber mosaic virus, the yield of the purified SSV-4-2A was 20 mg/kg of tobacco leaf tissues.

Serological relationships

A rabbit was immunized by injections with purified SSV-4-2A preparations. The anti-

Table 2. Differential reactions of soybean varieties and other plant species to Indonesian isolates and Japanese strains of soybean stunt virus

Family Plant species tested Variety	Symptoms							
	SSV-4-2A		SSV-13C		SSV-AE		SSV-B	
	IL	NIL	IL	NIL	IL	NIL	IL	NIL
Leguminosae								
Glycine max								
var. Tokachinagaha[1]	o	—	o	—	o	—	o	—
Hakuho No. 1[2]	o	—	o	—	o	—	o	(M)
Kariutakiya No. 28[3]	o	—	o	—	o	m	o	—
Dewamusume[4]	o	M	o	—	o	—	o	—
Shiratama No. 10[5]	o	M	o	—	o	—	o	M
Kwanggyo[6]	cs	M	o	M	o	—	cs	—
Nemashirazu[7]	o	M	o	M	o	m	o	—
York[8]	o	M	o	M	o	—	cs	M
Marshal[9]	o	M	o	M	o	m	cs	M
Shirotsurunoko[10]	o	N	o	N	o	m	o	M
Phaseolus vulgaris								
var. Black Turtle Soup	ns	—	o	—	o	—	o	m
Bountiful	ns	—	o	—	o	—	o	m
Ohtebo	ns	—	o	—	o	—	o	m
Top Crop	ns	—	o	—	o	—	o	m
Tsurunashi Kintoki	ns	—	o	—	o	—	o	m
Pino U. I. No. 111	cs	—	o	—	o	—	cs	m
Pisum sativum								
var. Kinusaya sanjunichi	o	—	o	(m, N)	o	(m, N)	o	—
Vicia faba								
var. Wase	o	—	ns	—	ns	—	o	—
Vigna unguiculata subsp. *sesquipedalis*								
var. Kurodane sanjaku	cs, ns	—	ns	—	ns	—	cs	M
V. unguiculata subsp. *unguiculata*								
var. Black eye	cs, ns	—	ns	—	ns	—	cs	M
Compositae								
Zinnia elegans	o	lat	o	—	o	—	o	lat

M: Mosaic, N: Necrosis.
Other abbreviations are the same as in Table 1.
Note: The same reactions were observed on the following varieties of soybean classified into 10 groups. 1) Harosoy, Peking, Kitamishiro and Isuzu, 2) non, 3) Fukusennari, 4) Tohshiken, 5) non, 6) Hwanggeumkong, Jangbaegkong and Suweon No. 120, 7) Fusanari and Suweon No. 105, 8) Kurokawaseioh, Suzuhime, Kingen No. 1, Davis and Ogden, 9) Buffalo, Rampage, L78-434, Hakuto, Okuhara No. 1, Yuzuru, Toyosuzu, Yamashirotama, Ou No. 13, Norin No. 4 and Norin No. 5, 10) Norin No. 2.

serum against SSV-4-2A had a titre of more than 1/1,024 when tested by ring interface precipitin tests.

Serological relationships among Indonesian isolates and a Japanese strain of SSV, two strains of cucumber mosaic virus (CMV), and a strain of peanut stunt virus (PSV) were tested in agar gel double diffusion tests. Two Indonesian isolates (SSV-4-2A and SSV-13C) and the Japanese strain (SSV-B) showed a reaction of identity using the antiserum against SSV-4-2A (Plate 3, a). SSV-4-2A reacted with antisera against SSV-4-2A, SSV-A[6], CMV-Y[10], CMV-P[9] and PSV[11] by form-

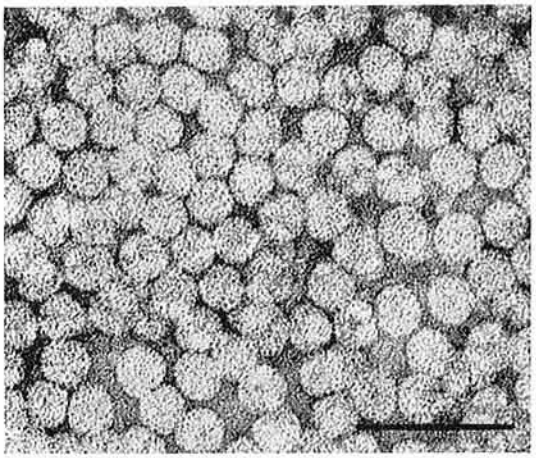

Plate 2. Electron micrograph of purified soybean stunt virus (SSV-4-2A) particles negatively stained with 2% uranyl acetate
Bar indicates 100 nm.

ing spurs with CMV-Y, CMV-P and PSV (Plate 3, b–f).

Transmission through seed

Mature seeds for seed transmission tests were harvested from soybean varieties, Toyosuzu and Okuhara No. 1, infected with SSV-4-2A or SSV-13C. Seed transmission was evaluated by the presence of symptoms and enzyme-linked immunosorbent assay (ELISA) of SSV in soybean cotyledons 10 days after sowing. Procedures for ELISA closely followed those developed by Clark and Adams[1].

The level of transmission of SSV-4-2A or SSV-13C through seeds of Toyosuzu and Okuhara No. 1 was more than 70%. The rate of seed transmission of Japanese strains of SSV was also over 70% in most varieties of soybean[2].

Discussion

Indonesian isolates and Japanese strains of SSV were serologically indistinguishable and were on the same level in transmission through soybean seeds.

Differential indicator plants of Indonesian and Japanese SSVs were soybean, French bean, pea, broad bean, asparagus bean, cowpea and *Zinnia elegans* (Table 2).

Based on pathogenicity and symptoms towards the differential soybean varieties for Japanese strains of SSV[3,6], two Indonesian isolates, SSV-4-2A and SSV-13C, were different from any Japanese strains of SSV (Table 3).

It is necessary to study the properties of the nucleic acid among SSV strains for classification.

Acknowledgement

The authors wish to express their sincere thanks to Dr. S. Takaya, a long-term expert to Japan-Indonesia Agricultural Research Strengthening Project (ATA-378 of JICA) at Central Research Institute for Food Crops in Indonesia, for his cooperative support and suggestions. They are indebted to Drs. H.

Table 3. Reactions of differential soybean varieties to Japanese strains and Indonesian isolates of soybean stunt virus

Differential variety	Reactions to strains and isolates of SSV						
	A	AE	B	C	D	13C	4-2A
Tokachinagaha	R	R	R	R	R	R	R
Ou No. 3	R	R	R	S	R	R	R
Harosoy	R	R	R	R	S	R	R
Nemashirazu	R	S	R	S	S	S	S
Kariutakiya No. 28	S	S	R	S	R	R	R
Norin No. 2	S	S	S	S	S(N)	S(N)	S(N)

R: Resistance, S: Susceptible, (N): Necrosis symptoms appeared.

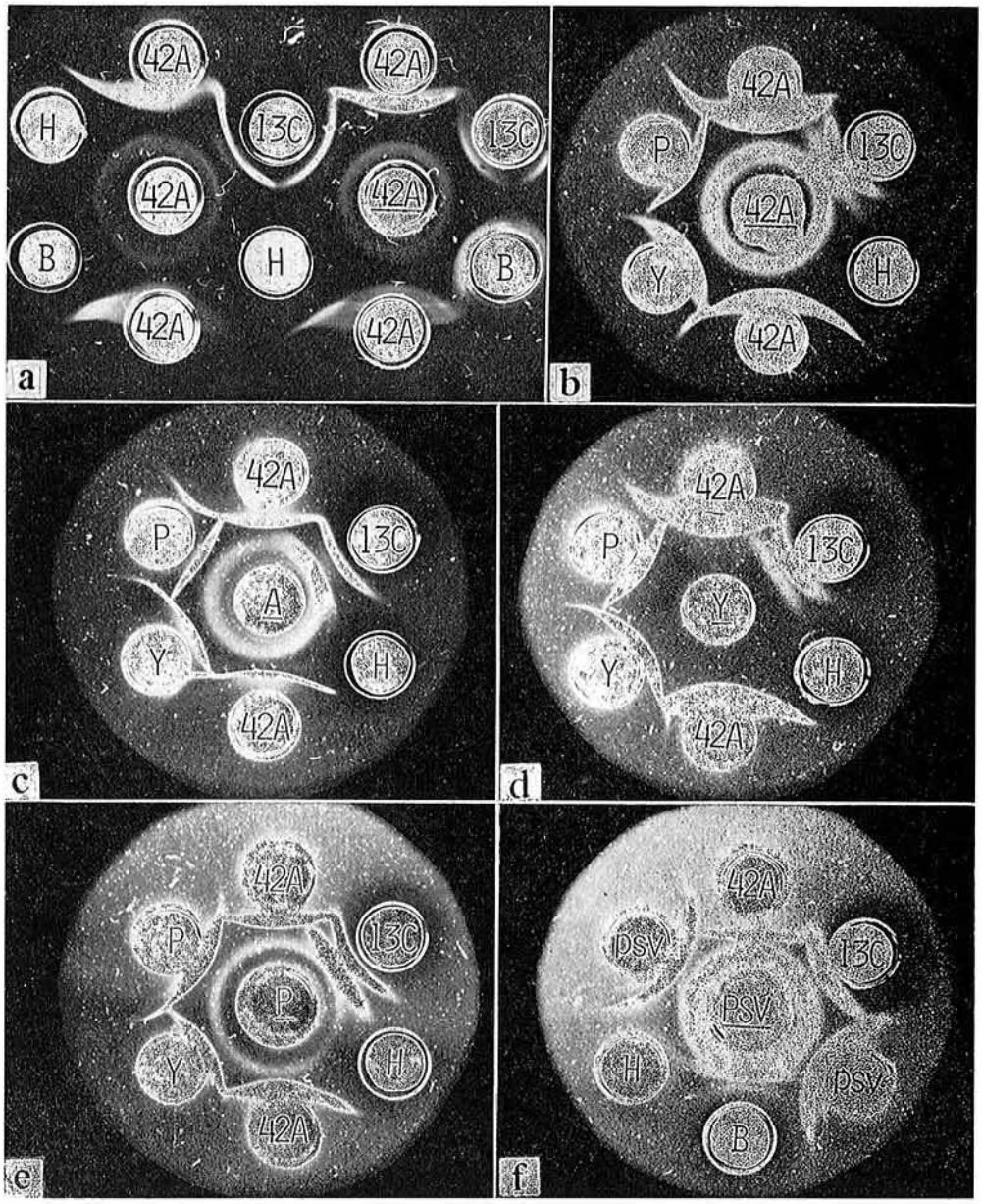

Plate 3. Agar gel double diffusion tests of soybean stunt virus (SSV-4-2A, SSV-13C and SSV-B), cucumber mosaic virus (CMV-Y and CMV-P) and peanut stunt virus (PSV)

Center well contains antisera against SSV-4-2A(42A), SSV-A(A), CMV-Y (Y), CMV-P(P) and peanut stunt virus (PSV).

Outer well contains antigens as follows:

42A=SSV-4-2A, 13C=SSV-13C, Y=CMV-Y, P=CMV-P, PSV=peanut stunt virus, and H=healthy crude sap from leaves of tabacco (Xanthi nc).

Tochihara and K. Hanada, National Agriculture Research Center in Japan for providing antisera to CMV-Y, CMV-P and SSV-A.

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(Received for publication, December 28, 1987)