

A Persistent Aphidborne Virus of Soybean, Indonesian Soybean Dwarf Virus

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ABSTRACT

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Indonesian soybean dwarf virus (ISDV) is distributed widely in Indonesia. Infected soybean plants show upward curling and rugosity of leaves and dwarfing with shortened petioles and internodes. ISDV infected only soybean among 22 plant species in six families tested and was transmitted by the aphid, *Aphis glycines* Matsumura, in a persistent manner but not by plant sap inoculation. Minimum acquisition and inoculation feeding periods by aphids for virus transmission were 6 hr and 1 hr, respectively. Partially purified virus preparations from infected soybean plants contained spherical particles, about 26 nm in diameter. In ultrathin sections, crystalline aggregates of spherical particles were observed in vacuoles of phloem cells of infected plants. ISDV did not show any relationship to soybean dwarf virus in serologic and cross protection tests.

Soybean (*Glycine max* Merrill) is one of the important secondary crops in Indonesia, and in recent years, plans have been made to increase production because soybean and its by-products are an important source of protein. Of the virus diseases that limit soybean production in Indonesia, soybean stunt and bean yellow mosaic virus are the two most important (9,10). The former is important because the causal virus is seed transmissible and widely distributed and the latter because the virus has a wide host range among Leguminosae and also is widely distributed.

In 1974, we observed a virus disease in many soybean fields in Java and Sumatra. The symptoms included dwarfing and were similar to those caused by the yellowing strain of soybean dwarf virus (SDV) in Japan (13).

Our results of experiments on symptomatology, vector specificity, host range, and serologic relationships indicate that the virus from Indonesia (ISDV) is distinct from SDV.

MATERIALS AND METHODS

Virus source. The virus source was a diseased soybean plant collected in 1974 from Cikeumeuh Experimental Field of the Central Research Institute for Agriculture, Bogor, Indonesia. The virus

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was identified at CRIA and at the Institute for Plant Virus Research, Ibaraki, Japan. The virus was maintained in soybean plants by successive aphid transmissions. SDV and the antiserum for these experiments were supplied by T. Tamada, Hokkaido Central Agricultural Experiment Station.

Virus transmission. Aphid transmission tests were done with virus-free aphids, *Aphis craccivora* Koch, *A. glycines* Matsumura, and *Aulacorthum solani* Kalt., reared on healthy peanut, soybean, and soybean plants, respectively.

Effects of the number of aphids on

transmission of the virus were determined by allowing groups of aphids a 2-day acquisition feeding on infected soybean plants. The aphids were then transferred to healthy soybean plants (5–20 aphids per plant) at the primary leaf stage and allowed a 2-day inoculation feeding. Feeding of aphids was terminated by spraying with an insecticide.



Fig. 2. Faint mottling and rugosity on trifoliate leaf of soybean infected with Indonesian soybean dwarf virus.



Fig. 1. Soybean infected naturally with Indonesian soybean dwarf virus.

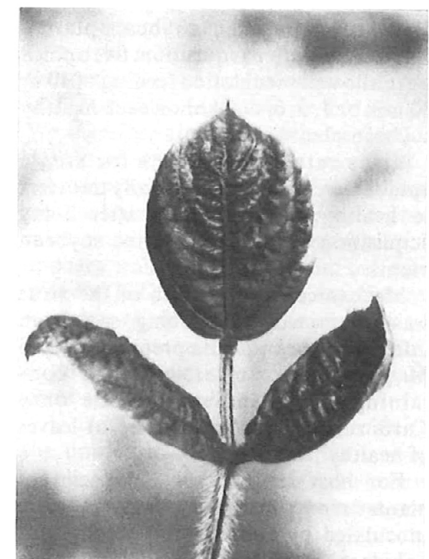


Fig. 3. Symptoms on young leaf of soybean infected with Indonesian soybean dwarf virus.

Table 1. Aphid transmission tests with Indonesian soybean dwarf virus

Soybean cultivar	Aphid	No. of plants infected/inoculated ^a
Okuharawase	<i>Aphis craccivora</i>	0/3
	<i>A. glycines</i>	6/13
	<i>Aulacorthum solani</i>	0/9
Shirotsurunoko	<i>A. glycines</i>	10/10
	<i>Aulacorthum solani</i>	0/3

^a Three-day acquisition and 3-day inoculation feeding periods and 5–20 insects per plant.

Table 2. Effect of the number of aphids (*Aphis glycines*) on the transmission of Indonesian soybean dwarf virus

Test	Aphids per plant					
	1	2	3	4	5	10
1	1/10 ^a	3/10	5/10	5/10	3/10	2/3
2	0/10	1/10	2/10	5/10	4/10	4/6

^a Numbers of plants infected/inoculated, after 2-day acquisition and 2-day inoculation feedings, on Davros soybean.

Table 3. Effect of acquisition and inoculation feeding periods on the transmission of Indonesian soybean dwarf virus by *Aphis glycines*

Test	Feeding period ^a						
	10 min	30 min	1 hr	3 hr	6 hr	24 hr	48 hr
Acquisition ^b							
1	...	0/10	0/10	0/10	0/10	4/10	8/10
2	...	0/10	0/10	0/10	1/10	2/10	6/10
Inoculation ^c							
1	0/10	0/10	1/10	1/10	4/10	7/10	...
2	1/10	0/10	1/10	2/10	4/10	3/10	...

^a Numbers of plants infected/inoculated using five aphids per Davros soybean plant.

^b Two-day inoculation feeding period.

^c Two-day acquisition feeding period.

For minimum acquisition feeding period tests, aphids were allowed an acquisition feeding of 30 min or 1, 3, 6, 24, or 48 hr on infected soybean plants. The inoculation feeding on healthy soybean plants was 2 days with five aphids per plant.

For the minimum inoculation feeding, aphids were allowed a 2-day acquisition feeding on infected soybean plants. Immediately after acquisition, five aphids were allowed inoculation feeding of 10 or 30 min or 1, 3, 6, or 24 hr on each healthy soybean plant.

Virus retention periods for single aphids were determined by daily transfers to healthy soybean plants after 2-day acquisition feeding on infected soybean plants.

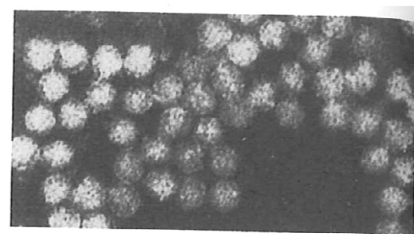
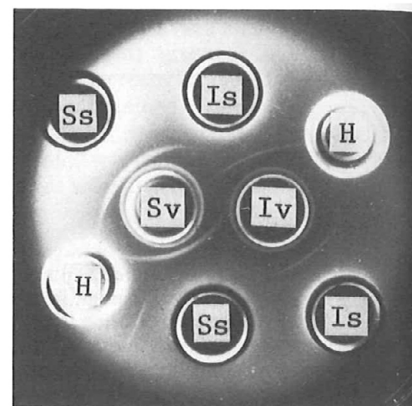
Mechanical transmission of the virus was attempted by rubbing sap from infected soybean plants prepared in 0.05 M phosphate buffer, pH 7.2, containing 0.02% sodium cyanide onto Carborundum-dusted (600 mesh) leaves of healthy test plants.

For host range tests, 22 species of plants representing six families were inoculated by allowing five viruliferous aphids a 2-day inoculation feeding on each seedling. Virus infection was usually confirmed by back inoculation to healthy

soybean plants with aphid vectors.

All inoculated plants were maintained in the greenhouse for at least 3 wk, during which plants were periodically sprayed with an insecticide.

Virus purification. The virus was partially purified according to the procedure of Takanami and Kubo (12) for tobacco necrotic stunt virus. Frozen, infected leaves and stems of soybean cv. Shirotsurunoko were ground in a meat grinder with 0.1 M citrate buffer, pH 6.0 (w/v), containing 1.5% Dricelase (Kyowahakko Co.), 0.1% thioglycolic acid, and 0.033 M Na-ethylenediaminetetraacetic acid (Na-EDTA). After incubation at 28–30 C for 1.2 hr, the homogenate was cooled in an icebath and expressed through cheesecloth. The expressed sap was mixed with 1/8 volume of chloroform and 1/15 volume of *n*-butanol, and the emulsion was broken by high-speed centrifugation (9,000 g, 20 min). The aqueous phase was centrifuged at 100,000 g for 70 min. Pellets were resuspended in 0.02 M phosphate buffer, pH 7.1, containing 0.002 M Na-EDTA, and centrifuged at 9,000 g for 10 min. The supernatant fluid was mixed with 0.25% Triton X-100 and subjected to one cycle of high-speed and ultracentrifugation as described. The resuspended pellets,

**Fig. 4.** Spherical particles in a partially purified preparation from soybean infected with Indonesian soybean dwarf virus**Fig. 5.** Reactions in immunodiffusion tests with Indonesian soybean dwarf and soybean dwarf viruses. Iv = Indonesian soybean dwarf virus, Sv = soybean dwarf virus, Is = antiserum against Indonesian soybean dwarf virus, Ss = antiserum against soybean dwarf virus, H = sap from healthy soybean leaf.

consisting of partially purified virus, were placed on 10–40% linear sucrose density gradients prepared with a gradient maker. Gradients were centrifuged for 150 min at 24,000 rpm using a Hitachi RPS 25 swinging-bucket rotor. After centrifugation, the opaque zone containing virus was removed with a syringe and centrifuged at 100,000 g for 70 min. The pellets were used for rabbit immunization and electron microscopy.

Serology. Rabbit immunization was done by four biweekly intramuscular injections of partially purified virus preparations emulsified with an equal volume of Freund's complete adjuvant. Antiserum was obtained from blood collected 20 days after the final injection. Serologic reactions were analyzed by double diffusion tests in 1% agar prepared in 0.02 M phosphate buffer, pH 7.1, containing 0.002 M Na-EDTA and 0.02% sodium azide. Reactions were allowed to develop at 10 C for 4 days before results were recorded.

Cross protection tests. In cross protection tests, soybean plants were inoculated with the first virus via aphids, and after symptoms appeared, the plants were inoculated with the second virus via aphids. Infection of the plants by the second virus was checked by the recovery tests to healthy soybean using the vector specific for the second virus 1 mo after the

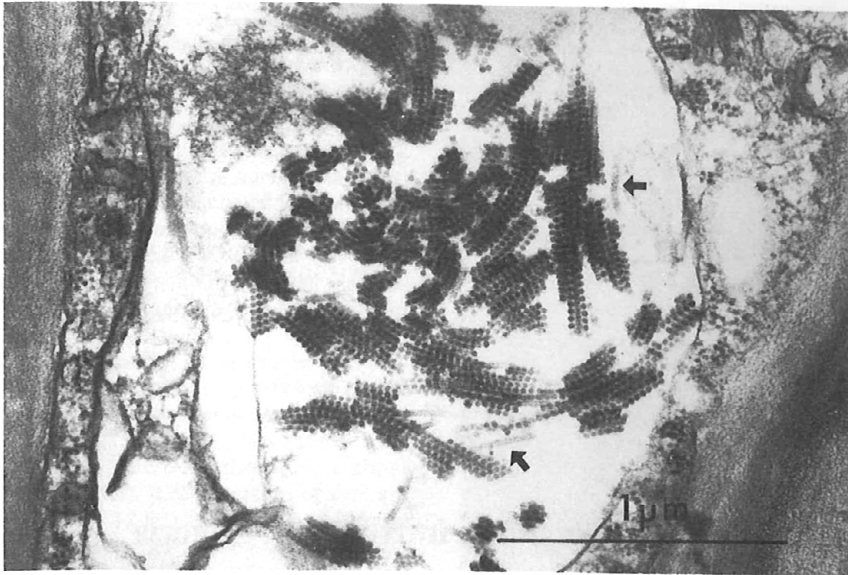


Fig. 6. Portion of a parenchyma cell in phloem tissue of a soybean leaf infected with Indonesian soybean dwarf virus. Arrows indicate viruslike particles and tubules.

second inoculation.

Electron microscopy. For ultrathin sections, small pieces of diseased soybean leaves were fixed with 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, for 1 hr and then postfixed with 1% osmium tetroxide in the same buffer for 2 hr. Fixed samples were dehydrated in an acetone series and embedded in epoxy resin. Ultrathin sections were cut using glass knives mounted on a Porter-Blum MT-2B ultramicrotome. Sections were stained with uranyl acetate and lead citrate and observed with a Hitachi Model H-500 electron microscope operating at 75 kV.

RESULTS

Symptomatology. Soybean plants naturally infected with ISDV showed dwarfing with shortened leaf petioles and internodes (Fig. 1) and had a slightly dark green color. The upper leaves were unusually small and curled upward; the lower leaves showed rugosity with shortened veins and interveinal white necrosis. Occasionally, these leaves had many holes because of their brittleness. Infected plants produced few pods.

In the greenhouse, cv. Davros soybeans that were inoculated in the primary leaf stage with ISDV by using viruliferous *A. glycines* developed a faint yellow-dark green mottling on the primary and first trifoliate leaves about 10 days after inoculation feeding. The second trifoliate leaves also showed faint mottling with occasional rugosity (Fig. 2). The upper leaves of these plants were unusually small and curled upward (Fig. 3). Later, the whole plant showed dwarfing and most of the infected plants produced no pods.

Virus transmission. ISDV was not mechanically transmissible when sap from diseased soybean leaves was used to

inoculate *Chenopodium amaranticolor*, *Gomphrena globosa*, *Arachis hypogaea*, *G. max*, *Lathyrus odoratus*, *Phaseolus vulgaris*, *Pisum sativum*, *Vigna radiata*, *V. sesquipedalis*, *V. sinensis*, *Nicotiana tabacum*, and *Petunia hybrida*.

ISDV was transmitted by *A. glycines*, but not by *A. craccivora* or *Aulacorthum solani*, when groups of 5–20 aphids were allowed 3-day periods of acquisition and inoculation feeding (Table 1).

Virus-vector relationships. Virus transmission rates were highest when more than three aphids were used per plant. However, transmission rates did not reach 100% even when 10 aphids were used per plant (Table 2).

The minimum acquisition feeding period by *A. glycines* was 6 hr, after which the percent transmission increased with increases in the acquisition period (Table 3). The minimum inoculation feeding period appeared to be 1 hr, although in some rare cases aphids could transmit the virus after a 10-min inoculation feeding. As the inoculation feeding period increased, the percent transmission increased (Table 3). The longest retention period of the virus in the aphid vector was 9 days. The aphids were also able to transmit the virus after molting.

Host range. The following plants were tested by using infective aphids and found not to be susceptible to ISDV: *C. amaranticolor*, *Brassica rapa*, *A. hypogaea*, *Astragalus sinicus*, *Crotalaria juncea*, *Lathyrus odoratus*, *Phaseolus vulgaris*, *Pisum sativum*, *Trifolium hybridum*, *T. incarnatum*, *T. pratense*, *T. repens*, *T. repens* f. *giganteum*, *Vicia faba*, *Vigna radiata*, *V. sesquipedalis*, *V. sinensis*, *Nicotiana tabacum*, *Petunia hybrida*, *Sesamum indicum*, *Cucumis sativus*, and *Cucurbita pepo*.

Virus purification. A single, opaque band was observed in sucrose density

gradients. This band contained spherical particles with an average diameter (30 particles) of 26 nm (Fig. 4) when observed under the electron microscope.

Serology. In immunodiffusion tests, both ISDV and SDV reacted positively with their antisera in the homologous combinations but not in heterologous combinations (Fig. 5).

Cross protection tests. In the cross protection tests between ISDV and SDV, challenge virus SDV infected 11 of 12 plants that had been infected with ISDV. Likewise, challenge virus ISDV infected two of 18 plants that had been infected with SDV. Soybean plants in the latter test were older because of the late appearance of SDV symptoms, making it very likely that the plants might have escaped infection by ISDV.

Electron microscopy. In ultrathin sections, aggregates of spherical viruslike particles and filamentous structures were observed in the vacuoles of phloem parenchyma cells of infected plants (Fig. 6). The particles were about 24 nm in diameter and often in crystalline arrays. The filamentous structures were 25–30 nm in diameter and had central electron translucent cores of about 5 nm in diameter. No viruslike particles were observed anywhere else in the infected tissue or in comparable healthy tissue.

DISCUSSION

The symptoms of ISDV infection in soybean and the transmission mode by aphids were similar to those of the yellowing strain of SDV in Japan. Because of these similarities, ISDV was at first named the Indonesian strain of SDV (1). However, our results clearly indicate that the vector species and host range of the virus in Indonesia differ from those of SDV in Japan. Moreover, serologic and cross protection tests offered no evidence of any relationship between the two viruses.

To our knowledge, only 10 viruses infect leguminous plants that have a persistent relationship with their aphid vectors, and none has been reported to be transmitted by *A. glycines* (2–8, 11, 14, 15). We therefore consider this to be the first report of *A. glycines* as a vector of a persistent aphidborne virus of leguminous plants. Moreover, all of these viruses can infect plant species other than soybean. It appears unlikely that the limited host range of ISDV found in our experiments was due to preferential feeding by *A. glycines* since the aphids did survive for 2 days of inoculation feeding on many plant species tested.

In view of the virus vector specificity, the limited host range, and the lack of serologic relationship to SDV, we believe that ISDV should be regarded as a new virus of soybean.

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