THE OCCURRENCE OF INDONESIAN SOYBEAN DWARF VIRUS ON SOYBEAN IN THAILAND

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Abstract

A virus transmitted by aphid, *Aphis glycines,* in a persistent manner was detected from dwarfed soybean plants with leaf rolling or rugose symptoms that were collected in Phitsanulok, northern Thailand in 1979. The virus was not transmitted by sap inoculation. Partially purified preparations from infected soybean plants contained spherical particles 25-30 nm in diameter, which reacted positively with antiserum to Indonesian soybean dwarf virus in agar gel double diffusion tests. Ultrathin sections of infected soybean leaves contained aggregations of virus-like particles in phloem cells similar to those of the Indonesian soybean dwarf virus. Based on these results, the virus was identified as Indonesian soybean dwarf virus.

1. Introduction

Many dwarfed soybean plants with leaf rolling or rugose symptoms were observed in northern and north-eastern Thailand. This disease was transmitted by grafting and aphid, *Aphis glycines,* but not by sap inoculation.

This paper describes the transmission and serological relationships of the virus isolated by aphid in a persistent menner.

2. Materials and methods

The virus used in this experiment was isolated from naturally infected soybean to soybean by aphid, *Aphis glycines,* in a persistent manner, and maintained by grafting or aphid transmission.

All the test plants were grown in a glasshouse. Sap inoculation was carried out by rubbing Carborundum (600 mesh)-dusted leaf surfaces with cotton pieces soaked in inoculum. Inoculum was prepared by grinding the diseased soybean leaves with 0.05 M phosphate buffer containing 10 mM diethyldithiocarbamate and 1 mM L-cysteine.

Aphid transmission was carried out using virus-free aphid, *Aphis glycines,* reared on healthy soybean plants.

Partial purification of the virus was conducted according to the purification procedure of tobacco necrotic stunt virus described by Takanami and Kubo (3). Frozen, infected leaves and stems of soybean cv. Shirotsurunoko were ground in a meat grinder with 0.1 M citrate buffer, pH 6.0, containing 1.5% Dricelase

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(Kyowahakko Co.), 0.1% thioglycolic acid, and 0.033 M sodium ethylenediaminetetra-acetic 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 above. The resuspended pellets were placed on 10–40% linear sucrose density gradients prepared with a gradient maker. Gradients were centrifuged for 150 min at 60,000 g using a Hitachi RPS-25 swinging bucket rotor. After centrifugation, the opaque zone containing the virus was removed with a syringe and centrifuged at 100,000 g for 70 min.

Serological relationships were analyzed by double diffusion tests in 1% agar prepared in 0.02 M phosphate buffer, pH 7.1, containing 2 mM Na-EDTA and 0.02% sodium azide.

In 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 post-fixed with 1% osmium tetroxide in the same buffer for 2hr. 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 examined under a Hitachi Model H-500 or H-300 electron microscope operating at 75 Kv.

3. Results

1) Symptoms

The diseased soybean plants showed dwarfing with shortened petioles and internodes, and sometimes a slightly dark-green color. The upper leaves of the diseased plants were very small and cupped up, and the lower leaves showed rugosity (Fig. 1).

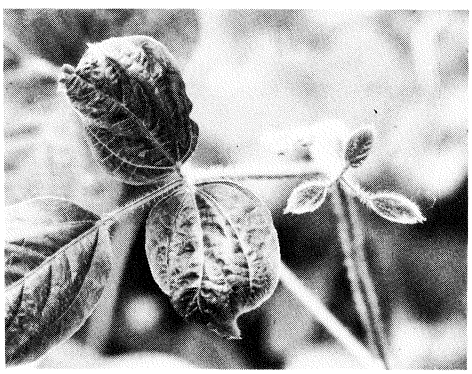

Fig. 1. Rugosity on small leaf of soybean infected with Indonesian soybean dwarf virus.

2) Transmission

1) Sap inoculation. The virus was inoculated mechanically to healthy soybean plants using sap of diseased soybean leaves. However, inoculated soybean plants did not show any symptoms.

2) Aphid transmission. Groups of aphids were allowed an acquisition access period of 2 days on diseased soybean plants, and each group of 10 aphids was transferred to a healthy soybean plant, and again transferred to a healthy plant every two days until six days. As shown in Table 1, 1/9, 3/9 and 3/9 of the test plants in the three transfers showed dwarfing symptoms, suggesting that the virus was transmitted by aphid in a persistent manner.

After an acquisition access of 2 days, single aphids were transferred daily to healthy soybean plants until 6 days. Eight insects among 11 aphids transmitted the virus, and some of them transmitted the virus in more than 2 plants (Table 2).

Table 1. Serial transmission tests of Indonesian soybean dwarf virus by *Aphis glycines*

Transfers	No. of infected plants /no. of inoculated plants
1	1 / 9
2	3 / 9
3	3 / 9

Acquisition and inoculation access periods: each 2 days,
Number of aphids per test plant: 10 insects.

Table 2. Daily transfer tests of Indonesian soybean dwarf virus by single *Aphis glycines* [a)]

Insect number	Transfer times [b)]					
	1	2	3	4	5	6
1	-	-	-	+	-	-
2	-	+	+	-	-	-
3	-	-	-	-	-	-
4	-	+	-	-	-	-
5	-	-	-	-	-	-
6	-	-	-	-	-	-
7	-	-	+	+	-	-
8	-	+	+	+	+	-
9	+	+	+	-	-	-
10	-	+	-	-	-	-
11	+	+	+	+	+	-

a): Preceded by 2 day acquisition access period.
b): Inoculation access period of 1 day.
+: symptoms appeared, -: symptoms did not appear.

3) Virus purification and serology

A single, opaque band was observed in sucrose density gradients. Purified samples contained spherical particles, 25-30 nm in diameter.

In agar gel double diffusion tests, the virus reacted positively with antiserum to the Indonesian soybean dwarf virus from Indonesia (2) (Fig. 2).

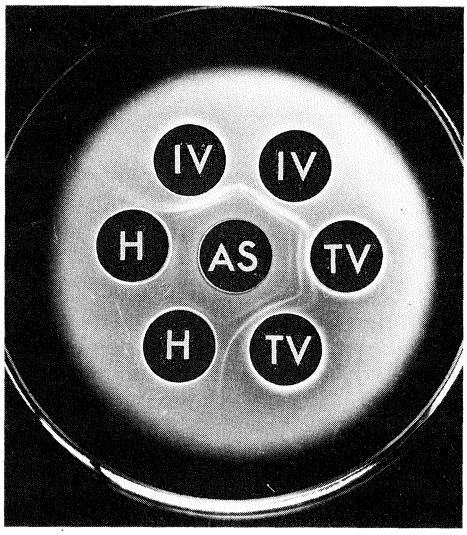

Fig. 2. Serological tests of the virus with Indonesian soybean dwarf virus from Indonesia in agar gel.
AS: antiserum to Indonesian soybean dwarf virus,
IV: Indonesian soybean dwarf virus from Indonesia,
TV: Indonesian soybean dwarf virus from Thailand,
H: Juice from healthy soybean leaves.

4) Electron microscopy

In ultrathin sections, aggregates of spherical viruslike particles were observed in the vacuoles of phloem parenchyma cells of infected plants. (Fig. 3).

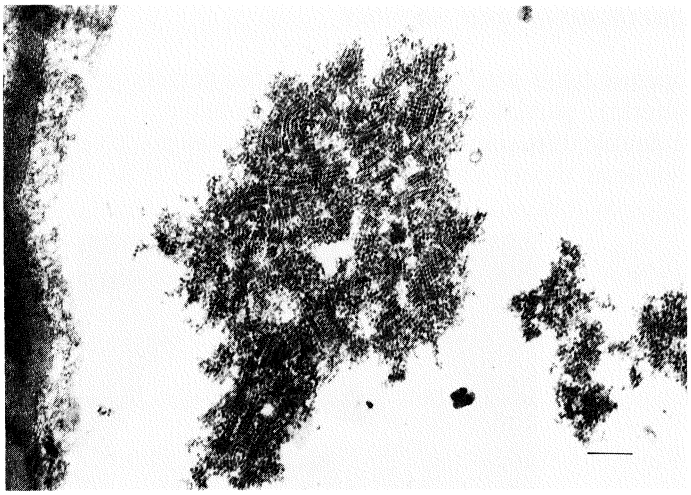

Fig. 3. Aggregations of virus-like particles in parenchyma cell in phloem tissue of soybean leaves infected with Indonesian soybean dwarf virus. Bar = 200 nm.

4. Discussion

The occurrence of Indonesian soybean dwarf virus (ISDV) on soybean was first reported from Indonesia in 1974 (2). The virus isolated from soybean in Thailand was identified as ISDV based on symptoms, vector species, virus-vector relationships, and serological relationships. This is the only report of the occurrence of the virus outside of Indonesia.

Since the host range of ISDV is limited to soybean only, it is likely that the virus disease may occur in areas where soybean is cultivated all the year round in the upland fields of tropical countries.

Soybean dwarf virus (SDV) (4), ISDV (2), milkvetch dwarf virus (MDV) (1) and cowpea stunt virus (CSV) (unpublished) are known as the causal viruses of dwarfing disease of soybean. SDV is transmitted by *Aulacorthum solani,* ISDV by *Aphis glycines,* and MDV and CSV by *Aphis craccivora*. The vector species of these diseases are very specific and very convenient for the identification of these diseases.

Aphis glycines is widely distributed in the world, therefore, ISDV may occur on soybean in areas where soybean is grown in all seasons.

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