# SOYBEAN YELLOW VEIN, A NEW VIRUS DISEASE OF SOYBEAN

# Toshihiro Senboku\*, Kruapan Kittipakorn\*\*, Surapee Kiratiyp-angul\*\*, Wanphen Srithongchai\*\*<sup>(1)</sup>, Pornpod Thongeerkom\*\*<sup>(2)</sup>, Nualchan Deema\*\*

#### ABSTRACT

A virus was isolated from soybean plants showing yellow vein symptoms that were collected in a field of an experimental station located in the central part of Thailand in 1982. The virus was a new virus of soybean based on particle morphology, host range, stability in crude sap, and so on. It was designated as "Soybean yellow vein virus" (SYVV). The occurrence and distribution of SYVV disease were limited to a field where soybean was cultivated in rotation with sorghum. Numerous rod-shaped virus particles (about 500–550 nm in length and 15–20 nm in width) were detected in the dip preparations of soybean leaves infected with SYVV. *Chenopodium amaranticolor* and *C. quinoa* showed distrinct chlorotic local lesions on the inoculated leaves, and only soybean was recognized as a systemic infection host of SYVV. *Aphis gluycines* (0/60) and *Bemisia tabaci* (5/50) failed to transmit SYVV. The dilution end point, thermal inactivation point and longevity in vitro of SYVV were,  $10^{-3} - 10^{-4}$ , 35–40°C (10 min) and 2–3 hours at 4°C respectively. Attempts to obtain a purified preparation of SYVV were made. After sucrose density gradient centrifugation, the peak fraction identified in the relative absorbance curve with ultra-violet light (254 nm absorbance zone) showed the highest infectivity and also contained numerous rod-shaped particles.

### Introduction

A virus was isolated mechanically from soybean plants showing yellow vein symptoms, that were collected in a field of Phraphutthabat Field Crop experimental Station located in the central part of Thailand in 1982. The occurrence and distribution of SYVV disease were limited to a field where soybean was cultivated in rotation with sorghum. The diseased soybean plants (cultivar SJ5) were collected from the field. The virus was a new virus of soybean based on particle morphology, host range, stability in crude sap, and so on. It was designated as "Soybean yellow vein virus" (SYVV). Results of studies on SYVV are presented in this paper.

# Materials, methods and results

The virus was isolated by sap inoculation from naturally infected soybean plants showing yellow vein symptoms, and maintained by sap inoculation or grafting for tests in the laboratory.

#### 1 Symptoms and host range

The inoculated soybean plants (cultivar SJ5) showed mottling or slight yellow banding symptoms on the upper leaves at three weeks after inoculation. Later the yellow vein symptoms in the infected plants, were more distinct and associated occasionally with vein necrosis (Fig.1).

<sup>\*</sup> Plant Pathologist Tropical Agriculture Research Center, Ministry of Agriculture, Forestry and Fisheries, Japan, Tsukuba Science City, Yatabe, Tsukuba, Ibaraki 305, Japan.

<sup>\*\*</sup> Plant Virology Group, Division of Plant Pathology and Microbiology, Department of Agriculture, Thailand. Bangkhen, Bangkok 10900, Thailand.

<sup>\*\* (1)</sup> Present address: University of Adelaide. Adelaide, South Australia, 5001 Australia.

<sup>\*\* (2)</sup> Present address: Thai Monsanto Chemicals Co. Ltd., Srilom, Bangrak, Bangkok 10500, Thailand.



Fig. 1 Symptom on soybean 'SJ5'.

Host range tests of SYVV were performed by sap inoculation to 16 plant species of 6 families and back inoculation to *Chenopodium amaranticolor* as the local lesion host of SYVV. The virus infected three plant species of two families (Table 1).

*Chenopodium amaranticolor* was used as a differential plant of the virus (Fig.2). Only soybean was recognized as a host showing systemic infection in host range tests. The reactions among three soybean cultivars, 'Tsurunoko', 'SJ4', and 'SJ5' were tested by sap inoculation of SYVV. 'Tsurunoko' was found to be the most susceptible soybean culvivar to SYVV (24% infection ratio).

Test plant		Symptom	Back inoculation
Family	Species	U I	C. amaranticolor
Amaranthaceae	Gomphrena globosa		
	Chenopodium amaranticolor	Chlorotic local lesion	/
	C. murale	-	-
	C. quinoa	Chlorotic local lesion	+
Cucurbitaceae	Cucumis sativus	-	
Leguminosae	Glycine max		
	(SJ1,4,5)	Yellow vein	+
	(Toyosuzu)	Yellow vein	+
	(Tsurunoko)	Yellow vein	+
	Vigna mungo	-	-
	V. radiata	-	-
	V. sesquipedalis	-	-
Pedaliaceae	Sesamum indicum	-	-
Solanaceae	Datura metel	-	-
	D. stramonium	-	-
	Nicotiana glutinosa	-	-
	N. tabacum	-	-
	Physalis floridana	-	-
	Solanum melongena	-	-

 Table 1
 Host range of soybean yellow vein virus

( ): cultivar.



Fig. 2 Chlorotic local lesion on Chenopodium amaranticolor.

### 2 Insect transmission

Transmissibility of SYVV by aphid (*Aphis glycines*) and whitefly (*Bemisia tabaci*) was tested. These insects were allowed an acquisition access of one week. Then, these insects were eliminated by spraying insecticide. In these experiments, *A. glycines* (0/60) and *B. tabaci* (0/50) failed to transmit SYVV.

#### 3 Multiplication and distribution of SYVV in soybean plant

Attempts to determine whether SYVV is capable of multiplying in the inoculated soybean plants were made in order to obtain suitable material for purification using *C. amaranticolor* as assay plant. After inoculation of SYVV to young seedlings (primary leaf stage) of 'Tsurunoko' soybean, the leaves of the inoculated soybean plant were harvested weekly as experimental material. These samples were inoculated to *C. amaranticolor*, and the number of local lesions on the inoculated leaves was counted 6 to 7 days later. The samples tested at three weeks after inoculation showed a high degree of infectivity. The distribution of SYVV in the 'Tsurunoko' soybean plants infected systemically was examined at three weeks after inoculation using *C. amaranticolor* as assay plant. The infected leaves of each 'Tsurunoko' soybean plant were divided into three samples (each including 1st to 3rd compound leaf respectively). The inoculated leaves could not be examined, because they fell off the plant before the tests were performed. From these results, SYVV appeared to be able to multiply actively in the upper leaves of the infected soybean plants at three weeks after inoculation.

#### 4 Electron microscopic observations

Attempts were made to detect the virus particles of the causal agent by electron microscopic observations using the dipping method and ultra-thin sections. Numerous rod-shaped virus particles (about 500–550 nm in length and 15–20 nm in width) were detected in the samples (Fig. 3, 4). Lumps of virus particles were also observed in the phloem and mesophyll parenchyma of the infected plants in the ultrathin sections (Fig. 5).



Fig. 3 Particles detected in dip preparation of soybean sample infected with SYVV (Bar represents 500 nm).



Fig. 4 Histogram of distribution of particle length of SYVV.



Fig. 5 Ultrathin section of soybean sample infected with SYVV (phloem parenchyma) (Bar represents 500 nm).

# 5 Stability in crude sap and purification of SYVV

Stability of virus in crude sap of soybean leaves infected with SYVV was tested by conventional procedures using *C. amaranticolor* as test plant. The dilution end point, thermal inactivation point and longevity *in vitro* of SYVV where  $10^{-3} - 10^{-4}$ ,  $35-40^{\circ}$ C (10 min) and 2–3 hr at 4°C, respectively. Attempts to obtain a purified preparation of SYVV were made according to the procedure shown in Fig. 6.

Frozen tissue (diseased soybean plant)

two time volume buffer 0.1 M borate bufer pH 8.5, 0.005 M cystein, 0.01 M TGA, 1% 2 m EtoH Maceration and squeezing Extract 20% CCl<sub>4</sub> treatment 3,600g 15 min Aqueous phase 4% PEG (# 6,000) treatment) (0.2 M Nacl) 1 hr. stirring, 1 hr incubation 8,000g 30 min Pellet resuspend 0.1 M borate buffer pH 8.5 0.005 M DIECA 1% 2 m EtOH 10% chloroform treatment 3,600g 15 min Aqueous phase 2% triton X-100 treatment Differential centrifugation 100,000g 2 hr8,000g 10 min Sucrose density gradient (10-40%) centrifugation 25,000 rph 2 hr (Spinco SW25) (ISCO Scanning) Virus fraction

# Fig. 6 Purification of SYVV.

Relative absorbance curve with ultraviolet light (254 nm absorbance zone) of the purified preparation showed the presence of a small peak. The peak fraction identified in the absorbance curve was the most infective fraction, and also contained numerous virus particles (Fig. 7).



Fig. 7 Purified SYVV particles (Bar represents 500 nm).

# Discussion

Occurrence and distribution of SYVV disease was found to be limited to areas in which soybean was cultivated in rotation with sorghum. It is thus necessary to conduct further surveys in soybean fields. The agents capable of transmitting the virus in the field remain unknown, and Aphis glycines and Bemisia tabaci failed to transmit SYVV. Some physical properties of SYVV were determined. The virus consists of elongated particles whose infectivity was found to be unstable. Attempts to purify the virus were carried out, but the purification procedure requires further improvement. Numerous virus particles which appeared to be rod-shaped, about 550 nm in length and 15-20 nm in width were detected by electron microscopy in dip preparations of the specimens. The structure of the particles of SYVV was similar to that of the particles of the tobamo virus group (Harrison et al., 1971), except that the particles were longer, in spite of an observation reporting a length of 500-600 nm in the latter group. One of the green algae virus, Chara corallina virus, consists of rod-shaped particles 532 nm in length (Skotnicki et al., 1976). It was suggested that the *Chara corallina* virus which shows characteristics similar to those of the tobamo virus group (especially the chemical constituents of the virus particles), may be a member of this group in spite of differences in the length of the particles. In some members of the tobamo virus group, such as peanut clump virus (Thouvenel et al., 1976), soil-borne wheat mosaic virus (Brakke, 1971), broadbean necrosis virus (Inoue and Nakasone, 1980), beet necrotic yellow vein (Tamada, 1975), potato mop-top virus (Harrison, 1974), transmissibility studies have been carried out. Further investigations about the soil transmissibility of SYVV and serological relationships among the viruses mentioned previously and SYVV, are underway.

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#### Discussion

- **Makkouk, K.M.** (ICARDA): It appears to me that the dimensions of the particles of the virus you described and the lack of insect transmission suggest that you are dealing with a potex virus. Why did you consider that this virus could be a tobamo virus?
- **Answer:** Based on the structure of the particles it appeared to be a tobamo virus particularly since the *Chara corallina* virus which shows chemical characteristics of the particles similar to those of tobamo viruses also consists of rod-shaped particles 532nm in length as in the case of soybean yellow vein virus.
- **Reddy, D.V.R.** (ICRISAT): The virus you describe may not be a potex virus since the latter consists of particles appearing as flexuous rods lacking a canal. Soybean yellow vein virus may be a tobamo virus except for the length of the particles. You should also determine that there is no end-to-end aggregation. I would like to add that peanut clump and wheat mosaic viruses are not tobamo viruses and will be assigned to a new group the "furoviruses" whose particles are rod-shaped with a fungal appearance.
- **Honda, Y.** (Japan): The particles of soybean yellow vein virus are different from those of peanut clump morphologically. They are similar to those of tobacco mosaic virus except for the length. The vector of soybean yellow vein virus may be a soil fungus.
- Answer: The possibility of soil transmission is being considered.

Abu Kassim, A.B. (Malaysia): Did you compare soybean yellow vein virus with Centrosema mosaic or Crotalaria yellow mosaic potexviruses which are also infectious to soybean?

Answer: No, I did not make any comparative studies.