SOYBEAN CRINKLE LEAF DISEASE OCCURRING ON SOYBEAN IN THAILAND[1)]

Mitsuro IWAKI[2)], Pornpod THONGMEEARKOM[3)], Yohachiro HONDA[4)], Nonglak SARINDU[5)], Nualchan DEEMA[5)], and Preecha SURIN[5)]

Abstract

Soybean crinkle leaf, a new whitefly-borne disease affected soybean in Thailand. Infected soybean plants showed twisting or curling of leaves with veinal enations on the undersurface of the leaves. The disease agent was transmitted by the whitefly, *Bemisia tabaci* Genn. in a persistent manner and by grafting, but not by aphids, inoculation of sap or through seeds of soybean. Single whiteflies did not transmit the disease agent and about 40 insects were necessary for achieving high transmission rates. Minimum acquisition and inoculation access periods ranged between 30 and 60 min and about 10 and 30 min, respectively. Latent period in the whitefly vector ranged between 8 and 10 hr, and retention period in the vector lasted 9 days. The disease affected 11 plant species in three families (Compositae, Leguminosae, and Solanaceae) after whitefly transmission. Purified preparations of the causal agent revealed the presence of a large number of geminate particles, and also aggregations of virus-like particles were observed in the nuclei of infected cells, suggesting that the causal agent of the disease is a possible member of geminivirus.

1. Introduction

During surveys of virus diseases of soybean in Thailand, we observed soybean plants with crinkle leaf and vein-enation symptoms in many soybean growing areas of the country. Preliminary tests in the laboratory revealed that the disease was transmitted by whiteflies. The transmission and host range of the disease were studied and it was concluded that the disease is a new disease of soybean.

2. Materials and methods

1) The disease The disease agent used in this experiment was isolated from a plant infected by whitefly transmission from a naturally infected soybean plant collected at Phitsanulok, Northern Thailand, in 1980, and subsequently maintained in soybean plants either by grafting or by whitefly transmission (5).

2) Transmission Inoculum for sap inoculation was prepared by grinding diseased leaves in 0.05 M phosphate buffer, pH 7.0 or 8.0, containing 10 mM sodium diethyldithiocarbamate (DIECA) and 1 mM L-cysteine or 20 mM sodium sulphite.

---

1) Part of the results on soybean crinkle leaf : a new whitefly-borne disease of soybean was reproduced from Plant Disease 67 : 546-548, 1983 with the permission of the American Phytopathological Society (September 13, 1983).

2) National Institute of Agro-Environmental Sciences, Kannondai, Yatabe, Tsukuba, Ibaraki, 305 Japan.
3) Department of Agriculture, Bangkhen, Bangkok 10900, Thailand, present address : Thai Monsanto, Bangkok, Thailand.
4) National Agriculture Research Center, Kannondai, Yatabe, Tsukuba, Ibaraki, 305 Japan.
5) Department of Agriculture, Bangkhen, Bangkok 10900, Thailand.

Healthy seedlings were inoculated by rubbing the carborundum-dusted leaves with a cotton swab dipped in the inoculum.

Non-viruliferous whiteflies, *Bemisia tabaci* Genn., were reared on healthy hibiscus (*Hibiscus* sp.) or tobacco (*Nicotiana tabacum* L.) and non-viruliferous aphids, *Aphis craccivora* Koch, *A. glycines* Matsumura, and *Myzus persicae* Sulzer, were reared on healthy broad bean, soybean, and turnip, respectively.

Transmission tests using aphids were carried out by first starving aphids for 2 hr in a glass beaker before allowing an acquisition access period of 15 min on the diseased plants. After the acquisition access, groups of 10 aphids were transferred to each healthy soybean seedling (10–14 days old) for an inoculation access period of 1 day which was terminated by spraying with insecticides. In other tests, aphid transmission was carried out without preacquisition starvation and a 1-day acquisition access period was allowed before inoculation access as described above.

Whitefly transmission efficiency of the disease agent was determined by first allowing adult whiteflies an acquisition access period of 2 days on infected soybean plants. After acquisition, one, 5, 10, 20, or 40 whiteflies were transferred to each healthy soybean seedling at the primary leaf stage and allowed an inoculation access period of 2 days.

Minimum acquisition or inoculation access period for whitefly transmission was determined as previously described (4). For the minimum acquisition access period, groups of 40 whiteflies were allowed an acquisition access period of 1/6, 1/2, 1, 3, 6, 24, or 48 hr on diseased plants, followed by an inoculation access period of 2 days on each healthy soybean seedling. Minimum inoculation access period was similarly determined using the reciprocal access periods.

Latent period in whiteflies was determined by first allowing whiteflies an acquisition access period of 3 hr on infected soybean plants. Immediately after the acquisition access, each group of the 40 whiteflies was allowed a serial inoculation access period of 1, 2, 2, 2, 2, and then 12 hr on each of a series of six soybean seedlings.

Retention period in whitefly was determined by allowing whiteflies an acquisition access period of 2 days on infected soybean plants. Thereafter each group of 40 whiteflies was transferred every day to each healthy soybean seedling for 18 days. Since the number of insects decreased after each transfer, insects in two or three groups were mixed at the fourth, eighth, ninth and fifteenth transfers. Transmission was terminated by spraying with insecticides. Test plants used in whitefly transmission tests were observed for symptoms for about 4 wk after inoculation.

Transmission of the disease agent through seeds of infected soybean plants was also tested. One hundred and seventy two seeds harvested from infected soybean plants grown in the greenhouse were germinated and the seedlings were observed for symptoms of crinkle leaf disease.

3) Host range Thirty-two plant species in 10 families were inoculated using viruliferous whiteflies (40–50 insects/plant). At least six plants were inoculated for each plant species tested. Infection in each plant was indexed by back-inoculation to soybean by whitefly transmission at about 4 wk after inoculation.

4) Purification and electron microscopy Procedure for the purification of the causal agent and electron microscopy followed that for mungbean yellow mosaic virus (3).

3. Results

1) Symptomatology

Infected soybean plants in the fields showed twisting or curling of leaves with veinal enations on the undersurface of the leaves (Fig. 1, 2). In addition, the foliage of the infected plants was dark green in color, which enabled infected plants to be distinguished from the near-by noninfected plants. In the greenhouse, infected soybean plants showed yellow netting of veins at 10-14 days after inoculation (Fig. 3). Thereafter, veinal enations appeared on the undersurface of the leaves, as well as cupping or twisting of leaves (Fig. 4).

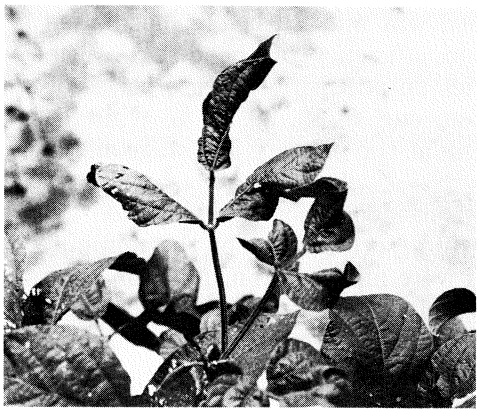

Fig. 1. Crinkle leaf symptoms on soybean infected with soybean crinkle leaf disease in field.

Fig. 2. Vein-enations on undersurface of soybean leaf infected with soybean crinkle leaf disease.

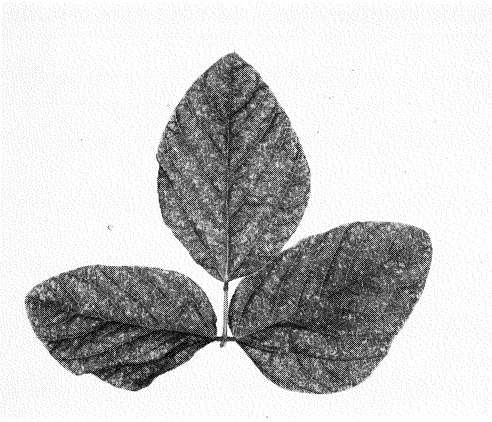

Fig. 3. Yellowing symptoms on netted vein of soybean leaf infected with soybean crinkle leaf disease.

Fig. 4. Crinkle leaf symptoms on soybean infected with soybean crinkle leaf disease in glasshouse.

2) Transmission

(1) Sap inoculation. Attempts to transmit the disease agent by mechanical inoculation were carried out from diseased soybean (*Glycine max*), bean (*Phaseolus vulgaris* cv. Top Crop), *Cassia tora,* tomato (*Lycopersicon esculentum*), *Datura stramonium* to soybean, Top Crop bean, *C. tora,* tomato, tobacco, *Nicotiana glutinosa,* petunia (*Petunia hybrida*). None of the plants inoculated showed any symptoms (Table 1).

Table 1. Sap inoculation tests of soybean crinkle leaf disease

Test	Source plant (inoculum)	Test plant	No. infected/inoculated plants
1	soybean	soybean	0/12
2	soybean	soybean	0/12
3	soybean	soybean	0/24
4	bean 'Top Crop'	soybean	0/ 6
5	bean 'Top Crop'	bean 'Top Crop'	0/30
6	*Cassia tora*	bean 'Top Crop'	0/12
7	*C. tora*	*C. tora*	0/ 4
8	tomato	tomato	0/ 6
9	bean 'Top Crop'	bean 'Top Crop'	0/20
10	bean 'Top Crop'	bean 'Top Crop'	0/26
11	tomato	tomato	0/18
12	datura	tomato	0/24
13	datura	tobacco	0/ 6
14	datura	*Nicotiana glutinosa*	0/ 6
15	datura	petunia	0/ 4

(2) Aphid transmission. Aphid transmission tests from soybean or tomato to soybean or tomato were performed in using three aphid species in a non-persistent or persistent manner.

None of the test plants exposed to aphids which had been previously allowed an acquisition access to diseased soybean plants became infected, regardless of whether non-persistent or persistent type of transmission tests were performed (Table 2).

Table 2. Aphid transmission tests of soybean crinkle leaf disease

Aphid	Diseased plant	Test plant	Fasting period	Acquisition access period	Inoculation access period	No. infected/ inoculated plants
Aphis craccivora	soybean	soybean	2 hr	15 min	1 day	0/6
A. glycines	soybean	soybean	2 hr	15 min	1 day	0/6
A. glycines	soybean	soybean	2 hr	1 day	2 days	0/6
Myzus persicae	tomato	tomato	2 hr	15 min	1 day	0/6
M. persicae	tomato	tomato	2 hr	1 day	2 days	0/6

No. of aphids per test plant: 10 insects.

(3) Whitefly transmission and causal agent-vector relationships. Single whiteflies were unable to transmit the disease agent but groups of 5, 10, 20, and 40 whiteflies transmitted it at the rates of 3/15, 4/15, 6/15, and 11/15, respectively

(Table 3).

Table 3. Effect of insect number on the transmission of soybean crinkle leaf disease by *Bemisia tabaci*

Number of insects per test plant	No. of infected /inoculated plants
1	0/14
5	3/15
10	4/15
20	6/15
40	11/15

Disease source plant and test plant: Shirotsurunoko soybean,
Acquisition and inoculation access periods: each 2 days.

The minimum acquisition access period for whitefly transmission ranged between 1/2 to 1 hr (Table 4). The minimum inoculation access period for whitefly transmission ranged between 1/6 to 1/2 hr (Table 5). The transmission rates increased with the increase in access periods for acquisition or inoculation.

Table 4. Effect of acquisition access period on the transmission of soybean crinkle leaf disease by *Bemisia tabaci*

Test	Acquisition access period						
	10 min	30 min	1 hr	3 hr	6 hr	24 hr	48 hr
I	0/15[a)]	0/15	1/15	1/12	7/15	8/15	11/15
II	0/ 9	1/11	0/15	4/15	9/15	13/15	12/15

Test plant: Yuzuru soybean, Inoculation access period: 2 days,
a): Number of infected plants/inoculated plants.

Table 5. Effect of inoculation access period on the transmission of soybean crinkle leaf disease by *Bemisia tabaci*

Test	Inoculation access period						
	10 min	30 min	1 hr	3 hr	6 hr	24 hr	48 hr
I	3/15[a)]	3/15	7/14	14/15	4/12	13/14	11/11
II	0/15	4/15	0/15	3/15	3/15	4/9	4/8

Test plant: Yuzuru soybean, Acquisition access period: 2 days,
a): Number of infected plants/inoculated plants.

Whiteflies required a period ranging between 8 to 10 hr after acquisition access before they could transmit the disease agent (Table 6) and could retain the transmission ability for at least 9 days after acquisition (Table 7).

(4) Seed transmission. None of the 172 seedlings which emerged from seeds harvested from infected soybean plants showed crinkle leaf symptoms.

Table 6. Latent period of soybean crinkle leaf disease in *Bemisia tabaci*

Group number of whiteflies	Hours after acquisition access						
	1	3	5	7	9	21	45
1	-	-	-	+	-	-	-
2	-	-	-	-	-	+	+
3	-	-	-	-	-	-	-
4	-	-	-	+	+	-	-
5	-	-	-	-	+	+	-
6	-	-	-	-	-	+	-
7	-	-	-	-	-	+	+
8	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-
10	-	-	-	-	-	-	+
11	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-

Acquisition access period: 3 hours,
40 whiteflies were used for the first transfer to each test plant.

Table 7. Retention of soybean crinkle leaf disease by *Bemisia tabaci* in successive daily transfers[a)]

Insect group number	Number of transfers										
	1	2	3	4	5	6	7	8	9	10	11-18
1	+[b)]	+	+	D							
2	+	-	-	D	+	+	-	-	+	-	-
3	+	+	+	D							
4	-	-	+	-							
5	+	-	D	-	+	+	-	-			
6	D	+	D	-					-	-	-
7	+	-	D	+							
8	-	-	D	-	+	-	-	-			
9	-	-	D	-							
10	+	-	-	D							
11	+	+	D	D	+	-	-	-	-	-	-
12	+	+	D	+							
13	-	+	-	-							
14	-	-	+	-	-	-	-	-	-		
15	-	-	-	-						-	-
16	-	-	D	-							
17	-	-	+	+	-	-	+	+	-		
18	+	+	+	+							
19	-	-	-	+							
20	+	+	+	+	-	-	-	-	+	-	-
21	+	+	+	-							
22	-	-	+	-							
23	-	+	-	+	-	-	-	-	+	-	-
24	-	+	-	+							

a): Acquisition access period = 2 days; number of insects per plant in first transfer = 40.
b): + = Plant infected, - = plant not infected, and D = test plant died.

3) Host range

The disease infected 11 plant species in three families via whitefly (Table 8). The following species and cultivars were infected systemically and showed distinct symptoms : *Cassia tora, Datura stramonium* (Fig. 5), *Glycine max, Lycopersicon esculentum, Nicotiana clevelandii, N. debneyi, N. glutinosa, N. tabacum, Petunia hybrida, Phaseolus vulgaris* cv. Top Crop (Fig. 6), and *Zinnia elegans* (Fig. 7). These plants showed vein clearing symptoms at about 10 to 14 days after inoculation by whiteflies and later showed leaf-curl or crinkle leaf symptoms.

The following species and cultivars were non-susceptible : *Arachis hypogaea,*

Brassica rapa, Cajanus cajan, Calendula arvensis, Capsicum annuum, Celoisia cristata, Chenopodium amaranticolor, C. quinoa, Cucumis sativus, Cucurbita pepo, Dolichos lablab, Gomphrena globosa, Gossypium hirsutum, Hibiscus esculentus, Morus bombycis, Phaseolus lunatus, P. vulgaris cv. Tsurunashi Kintoki, *Sesamum indicum, Solanum melongena, Tetragonia expansa, Trifolium pratense, T. repens, Vigna mungo, V. radiata, V. sesquipedalis,* and *V. unguiculata.*

Table 8. Host range of soybean crinkle leaf disease transmitted via *Bemisia tabaci*

Plant	Reaction and symptoms
Chenopodium amaranticolor	—
C. murale	—
C. quinoa	—
Celosia cristata	—
Gomphrena globosa	—
Brassica rapa	—
Arachis hypogaea	—
Cajanus cajan	—
Cassia tora	VC
Dolichos lablab	—
Glycine max	CL
Phaseolus lunatus	—
P. vulgaris cv. Kintoki	—
" cv. Top Crop	VC, LC
Trifolium pratense	—
T. repens	—
Vigna mungo	—
V. radiata	—
V. sesquipedalis	—
V. unguiculata	—
Capsicum annuum	—
Datura stramonium	VC, LC
Lycopersicon esculentum	VC, LC
Nicotiana clevelandii	VC, LC, Y
N. debneyi	VC
N. glutinosa	VC, LC
N. tabacum cvs. Blight Yellow and Xanthi	VC, LC
Petunia hybrida	VC, LC
Solanum melongena	—
Gossypium hirsutum	—
Hibiscus esculentus	—
Morus bombycis	—
Sesamum indicum	—
Cucumis sativus	—
Cucurbita pepo	—
Calendura arvensis	—
Zinnia elegans	VC

VC: vein clearing, CL: crinkle leaf, LC: leaf curling, Y: yellows, -: no infection.

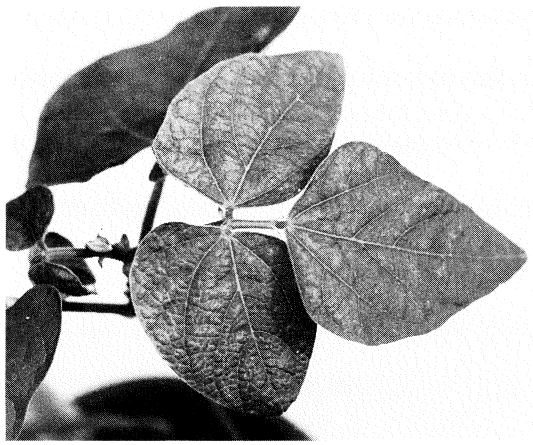

Fig. 5. Vein-clearing and mottle symptoms on Top Crop bean leaves infected with soybean crinkle leaf disease.

Fig. 6. Vein-clearing and leaf curling symptoms on *Datura stramonium* infected with soybean crinkle leaf disease.

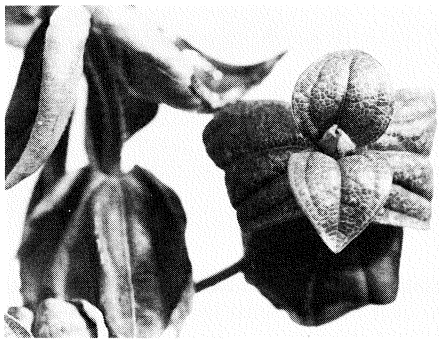

Fig. 7. Vein-clearing symptoms on *Zinnia elegans* infected with soybean crinkle leaf disease.

4) Purification and electron microscopy

Partially purified preparations of the causal agent from infected soybean and tomato included many virus-like geminate particles, but these preparations did not show the infectivity by whitefly transmission in applying the membrane feeding method (Fig. 8).

In ultrathin sections of infected soybean tissues, aggregations of virus-like particles were observed in the nuclei of infected cells (Fig. 9).

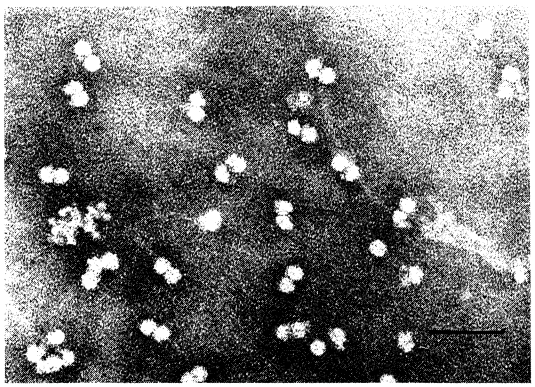

Fig. 8. Virus-like particles in purified preparation of soybean crinkle leaf disease. Bar = 100 nm.

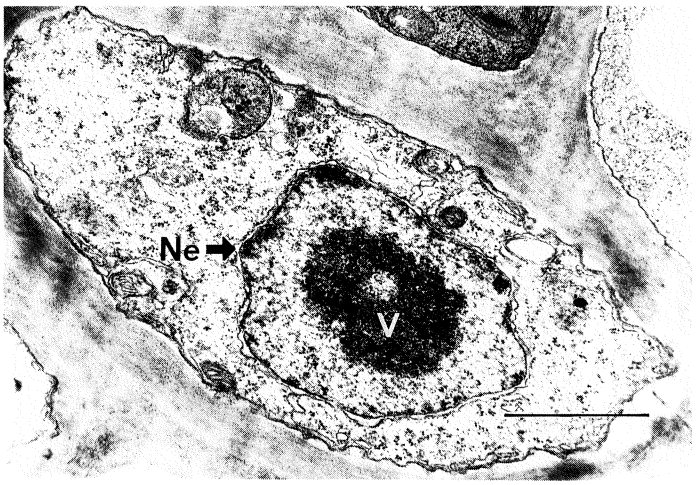

Fig. 9. Aggregation of virus-like particles (V) in nucleus of phloem cell of soybean leaf infected with soybean crinkle leaf disease.
Ne: nuclear envelope, Bar = 1,000 nm.

4. Discussion

Evidence from our studies on the symptomatology, transmission, and host range of the disease agent suggests that soybean crinkle leaf disease (SCLD) is a new whitefly-borne disease. The host range of SCLD is considered to be wide since many plant species other than the family Leguminosae were infected.

Although more studies should be carried out, we believe that the causal agent of SCLD may be a geminivirus.

The whitefly-borne diseases affecting soybean which are known to us include abutilon mosaic (2), Jatropha mosaic (1), mungbean yellow mosaic (5), and Rhynchosia mosaic disease (1). These diseases induce mosaic-type symptoms in infected host plants under either natural or experimental conditions.

The occurrence of SCLD in the fields was sporadic, but the disease was detected in most soybean growing areas of Thailand. In the country, whitefly-borne diseases are common probably due to the abundant whitefly vector population, and the continuous cropping systems adopted in most parts of country.

Acknowledgement

We would like to thank D. Hollis, Department of Entomology, British Museum (Natural History), London, England, and Y. Miyatake, Natural History Museum, Osaka, Japan, for identifying the whitefly.

Literature cited

1. Bird, J., Sanches, J., Rodrigues, R.L., and Julia, F. J. (1975). Rugaceous (whitefly-transmitted) viruses in Puerto Rico. Tropical Diseases of Legumes. Edited by Bird, J. and Maramorosch, K. pp 3-25.
2. Costa, A.S. (1975). Increase in the populational density of *Bemisia tabaci,* a threat of widespread virus infection of legume crops in Brazil. Tropical Diseases of Legumes. Edited by Bird, J. and Maramorosch, K. pp 27-49.
3. Honda, Y., Iwaki, M., Saito, Y., Thongmeearkom, P., Kittisak, K., and Deema, N. (1983). Mechanical transmission, purification, and some properties of whitefly-borne mungbean yellow mosaic virus in Thailand. Plant Disease 67 : 801-804.
4. Iwaki, M., Thongmeearkom, P., Prommin, M., Honda, Y., and Hibi, T. (1982). Whitefly transmission and some properties of cowpea mild mottle virus on soybean in Thailand. Plant Disease 66 : 365-368.
5. Thongmeearkom, P., Kittipakorn, K., and Surin, P. (1981). Outbreak of mungbean yellow mosaic disease in Thailand. Thia J. Agric. Sci. 14 : 201-206.