Soybean Crinkle Leaf: A New Whitefly-Borne Disease of Soybean

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ABSTRACT

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Soybean crinkle leaf disease occurred on soybean in many growing areas of Thailand. The disease agent was transmissible by the whitefly, *Bemisia tabaci*, in a persistent manner and by grafting but not by aphids, inoculations of sap, or through seeds of soybean. Single whiteflies did not transmit the disease agent and about 40 insects were necessary to achieve high transmission rates. Minimum acquisition and inoculation access times ranged from 30 to 60 min and from about 10 to 30 min, respectively. The latent period in the whitefly vector ranged between 8 and 10 hr, and the retention period in the vector lasted 9 days. The disease affected 11 plant species in three families (Compositae, Leguminosae, and Solanaceae).

Whitefly-borne plant diseases were known for many decades before the causal viruses were isolated and unequivocally characterized. Thus far, all viruses isolated from these diseased plants belong to one of two groups of plant viruses: the geminiviruses, which have paired or geminate particles, or rod-shaped particles, which have various length classes (4). Whitefly-transmitted geminiviruses have common characteristics such as an ss-DNA genome and the ability to induce nucleopathic changes in infected plants (4). Available evidence indicates that one member of the rod-shaped group has a ds-DNA genome (7), whereas other members have an ss-RNA genome (5).

During our studies on the identification 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 studies in the laboratory revealed that the disease was whitefly-borne. Therefore, we conducted transmission and host range studies and concluded that the disease reported is a new one.

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MATERIALS AND METHODS

Disease source and maintenance. The 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 (9). All test plants were grown in a glasshouse at Ibaraki, Japan, or in an insectproof house at Bangkok, Thailand.

Host range. Thirty-two plant species in 10 families were inoculated using viruliferous whiteflies (40–50 insects per 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 about 4 wk after inoculation.

Transmission. Inoculum for sap inoculation was prepared by grinding diseased leaves in 0.05 M potassium and sodium phosphate buffer, pH 7.0 or 8.0, containing 10 mM sodium diethyldithiocarbamate (DIECA) and 1 mM L-cysteine or 20 mM sodium sulphite. Healthy seedlings were inoculated by rubbing the Carborundum-dusted leaves with a cotton swab dipped in the sap.

Nonviruliferous whiteflies, *Bemisia tabaci* Genn., were reared on healthy hibiscus (*Hibiscus* sp.) or tobacco (*Nicotiana tabacum* L.) and nonviruliferous 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 an acquisition access period of 15 min on the diseased plants. After acquisition access, 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 before.

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, 1, 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.

The minimum acquisition or inoculation access period for whitefly transmission was determined as described previously (5). 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. The minimum inoculation access period was determined similarly, using the reciprocal access periods.

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

The 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 daily to each healthy soybean seedling for 18 days. Because the number of insects decreased after each transfer, insects in two or three groups were mixed at the fourth, eighth, ninth, and 15th 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 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.

RESULTS

Symptomatology and host range. Infected soybean plants in the fields showed twisting or curling of leaves with veinal enations on the undersurfaces of the leaves (Fig. 1A). In addition, foliage of infected plants was dark green, which enabled infected plants to be distinguished from nearby uninfected plants. In the greenhouse, infected soybean plants showed yellow netting of veins at 10–14 days after inoculation (Fig. 1B). Thereafter, symptoms included veinal enations on the undersurfaces of the leaves (Fig. 1C) and cupping or twisting of leaves (Fig. 1D).

The following species and cultivars were infected by whitefly transmission and showed distinct symptoms: *Cassia tora, Datura stramonium, Glycine max, Lycopersicon esculentum, Nicotiana clevelandii, N. debneyi, N. glutinosa, N. tabacum, Petunia hybrida, Phaseolus vulgaris* 'Top Crop,' and *Zinnia elegans.* These plants showed veinclearing symptoms at about 10–14 days after inoculation by whiteflies and later showed leaf-curl or crinkle leaf symptoms.

The following species and cultivars were not 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, Moros bombycis, Phaseolus lunatus, P. vulgaris* 'Tsurunashi Kintoki,' *Sesanum indicum, Solanum melongena, Tetragonia expansa, Trifolium pratense, T. repens, Vigna mungo, V. radiata, V. sesquipedalis,* and *V. unguiculata.*

Sap transmission tests. Attempts to transmit the disease agent by mechanical inoculation failed. None of the 48 soybean plants inoculated with sap prepared from infected soybean plants became infected. In another experiment, mechanical transmission of the disease agent from Top Crop bean (*Phaseolus vulgaris*), tomato (*Lycopersicon esculentum*), *Datura stramonium*, and *Cassia tora* previously infected via whitefly transmission was tested against the respective species, but none of them became infected.

Aphid transmission tests. None of the test plants exposed to aphids previously allowed acquisition access to diseased soybean plants became infected, regardless of whether nonpersistent or persistent types of transmission tests were performed.

Whitefly transmission and relationship with the causal agent. In preliminary tests, *B. tabaci* were able to transmit the causal agent of the crinkle leaf disease. Infected plants showed crinkle leaf symptoms at about 2 wk after inoculation access.

In transmission efficiency tests, single whiteflies were unable to transmit the disease agent but groups of 5, 10, 20, and 40 whiteflies transmitted it at rates (infected/inoculated plants) of 3/15, 4/15, 6/15, and 11/15, respectively. The minimum acquisition and inoculation access periods by whitefly transmission ranged between one-half to 1 hr and one-sixth to one-half hour, respectively, and transmission rates increased with the increase in access periods for acquisition or inoculation (Table 1). Whiteflies required a period of 8–10 hr after acquisition access before they could

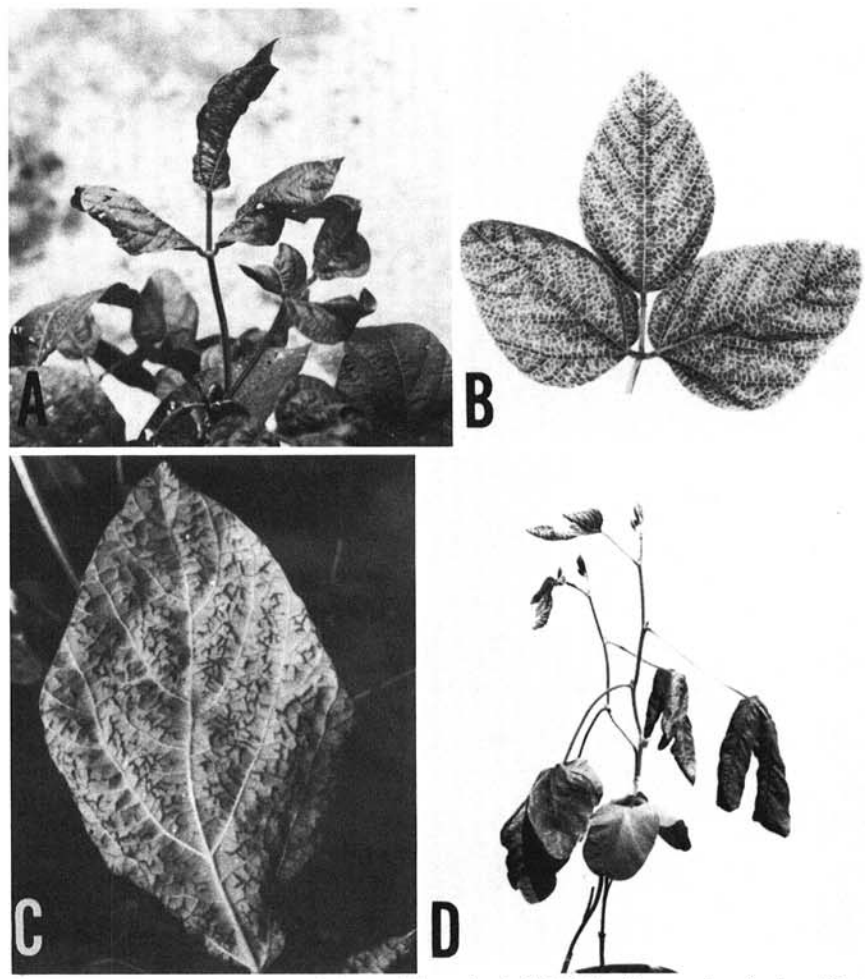

Fig. 1. (A) Symptoms of soybean crinkle leaf disease in the field, **(B)** yellow netting of veins, **(C)** vein enations on the undersurface of the soybean leaf, and **(D)** crinkle leaf symptoms in soybean.

Table 1. Acquisition and inoculation threshold periods for transmission of soybean crinkle leaf disease agent to Yuzuru soybean plants, using *Bemisia tabaci*[a]

Test	Access period						
	10 Min	30 Min	1 Hr	3 Hr	6 Hr	24 Hr	48 Hr
Acquisition[b]							
1	0/15[c]	0/15	1/15	1/12	7/15	8/15	11/15
2	0/9	1/11	0/15	4/15	9/15	13/15	12/15
Inoculation[d]							
1	3/15	3/15	7/14	14/15	4/12	13/14	11/11
2	0/15	4/15	0/15	3/15	3/15	4/9	4/8

[a] 40 insects per plant.
[b] Followed by 2-day inoculation access period.
[c] Number of plants infected/number of plants inoculated.
[d] Preceded by 2-day acquisition access period.

Table 2. 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.

transmit the disease agent and retain transmission ability for at least 9 days after acquisition (Table 2).

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

DISCUSSION

Evidence from our studies on the symptomatology, transmission, and host range of the disease suggests that soybean crinkle leaf disease (SCL) is a new whitefly-borne disease. Costa (3) divided the symptoms induced in plants affected by whitefly-borne diseases into three main types, yellow mosaic, yellowing, and leaf-curl types. Symptoms of SCL resemble those of the leaf-curl type, which consist of abnormal or unbalanced leaf growth as exemplified by the leaf-curl symptoms and enations observed in tobacco infected with tobacco leaf curl virus (8). The whitefly-borne diseases affecting soybean that are known to us include abutilon mosaic (2), Jatropha mosaic (1), mungbean yellow mosaic (9), and Rhynchosia mosaic diseases (1). These diseases induce mosaic-type symptoms in infected host plants under either natural or experimental conditions and occur naturally in the central part of South America, except mungbean yellow mosaic, which occurs in Asia (6). The host range of SCL is considered to be wide because many plant species other than the family Leguminosae were infected.

Although more studies should be carried out, we believe the causal agent of SCL may be a geminivirus. Our preliminary attempts to purify the causal agent from infected tissue yielded inconclusive and inconsistent results. Some geminate particles with the outline and size of geminiviruses, however, were detected in purified preparations when observed under the electron microscope. Purification of the causal agent was not easily accomplished because of the apparent low titer of the agent in infected tissue and difficulty in obtaining a sufficient amount of tissues infected via whitefly transmission.

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

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