

## STUNT OF ASPARAGUS BEANS INDUCED BY COWPEA STUNT VIRUS IN THAILAND

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### Abstract

A virus recovered from asparagus bean in Thailand was transmitted only to leguminous plants, including asparagus bean, cowpea, soybean, bean, pea, winged bean, and mungbean. The virus caused stunt with small leaves and shortened internodes in infected plants, and was transmitted by the aphid, *Aphis craccivora*, in a persistent manner, but not by mechanical inoculation. The minimum acquisition feeding periods by aphid for virus transmission were 24 hr, and the longest retention periods of the virus in the aphid were 8 days. Electron microscopic observation of ultrathin sections or leaf-dip preparations from the diseased asparagus bean did not reveal any virus-like particles. Based on these results, the virus was identified as cowpea stunt virus.

### 1. Introduction

Stunt of asparagus bean (*Vigna sesquipedalis*) and cowpea (*Vigna sinensis*) induced by cowpea stunt virus (CSV) was first identified in Indonesia in 1975 (1). CSV was transmitted by *Aphis craccivora* in a persistent manner, but not by sap inoculation. The particles of CSV have not been visualized. A similar virus disease affecting asparagus bean was found in Thailand in 1980. The present investigation was initiated to study various properties of CSV obtained from asparagus bean in Thailand, including host range and transmission by aphids.

### 2. Materials and methods

**1) Virus source** The virus used in this study was tentatively designated as an asparagus bean isolate of CSV (CSV-A). It was isolated from a diseased asparagus bean plant collected from the field in Thailand in 1980. The virus was maintained in asparagus bean plants by successive aphid transmissions.

**2) Insect transmission** For aphid transmission studies, three aphid species, *Myzus persicae*, *Aulacorthum solani*, and *Aphis craccivora* were used. Apterous aphids were allowed an acquisition access of 1-2 days on asparagus bean plants infected with CSV-A. Then, groups of five aphids were transferred to an individual plant of asparagus bean; 2 days later the aphids were killed with insecticides.

**3) Mechanical inoculation** Sap inoculation was performed by rubbing the Carborundum-dusted leaves of test plants with a piece of cotton soaked in a

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homogenate of infected leaves prepared in 0.1 M phosphate buffer containing 0.1% thioglycolic acid, pH 7.0.

**4) Electron microscopy** For electron microscopic observation, small pieces of infected asparagus bean leaves were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0, for 3 hr at 4°C. After dehydration with an acetone series, they were embedded in Spurr resin. The sections were stained with uranyl acetate and lead citrate. Leaf-dip preparations were made from infected asparagus bean leaves or petioles in 1% phosphotungstic acid, pH 7.0, or in 1% uranyl acetate. Some leaves were fixed in 10% formaldehyde, pH 7.0, or 3% glutaraldehyde before staining.

### 3. Results

#### 1) Host range

CSV-A was transmitted by *A. craccivora* from diseased asparagus bean plants to *Vigna sesquipedalis*, *Vigna sinensis*, *Glycine max*, *Phaseolus vulgaris*, *Pisum sativum*, *Psophocarpus tetragonolobus*, and *Vigna radiata*. Infected plants showed stunt with small and malformed leaves, shortened internodes, and many axillary buds.

Identification of susceptible lines of asparagus bean as test plants of CSV-A was carried out. No remarkable difference in the tested asparagus bean lines was observed (Table 1).

**Table 1. Reactions of some lines of asparagus bean to an asparagus bean isolate of cowpea stunt virus.**

Lines of asparagus bean	No. of aphids <sup>a)</sup>	
	5	10
Kanchanaburi	13/40 <sup>b)</sup>	6/19
BB PT-11	14/40	7/19
K2, 1A-60	15/40	7/19
K2, 1A-72	8/40	2/20
K2, 1B	7/40	3/20
75 cm	13/40	8/20

a) Number of aphids allowed an inoculation access to an individual plant.

b) No. of infected plants/no. of inoculated plants.

#### 2) Aphid transmission

CSV-A was transmitted by *Aphis craccivora* but not by *Myzus persicae* and *Aulacorthum solani*.

Groups of five *A. craccivora* fed on the infected plant for periods of 5 min, 1 hr, 7 hr and 24 hr to determine the feeding time necessary to acquire CSV-A. Table 2 showed that the minimum acquisition feeding period by *A. craccivora* was 24 hr.

Five viruliferous *A. craccivora* were taken from an infected colony and were

**Table 2. Change of infectivity with acquisition time, using 5 *Aphis craccivora* per indicator plant.**

Acquisition feed	Result
5 min	0/8 <sup>a)</sup>
1 hr	0/8
7 hr	0/8
24 hr	6/8

a) Numerator = plants infected; denominator = plants inoculated.

transferred daily to fresh seedlings of asparagus bean. Twenty seven out of the 150 aphid groups were able to transmit CSV-A. The longest retention period of the virus in the aphid vector was 8 days. The results of these experiments left no doubt that CSV-A was a persistent type virus.

### 3) *Electron microscopy*

Electron microscopic observation of leaf-dip preparations and ultrathin sections from CSV-A infected leaves of asparagus bean plants did not reveal any virus-like particles.

### 4) *Purification*

Purification of CSV-A from infected leaves of asparagus bean plants was attempted according to the procedure developed by Tsuchizaki *et al.* (3) or Tamada (2). No purified virus was obtained by this procedure.

## 4. Discussion

On the basis of host range, symptomatology, and aphid transmission mode the virus appeared to be a strain of CSV. CSV is a persistent aphid-transmitted virus widely distributed in Indonesia (1). The virus is similar in aphid transmission to a number of other persistent aphid-transmitted viruses including soybean dwarf virus, Indonesian soybean dwarf virus, milk-vetch dwarf virus, pea leafroll virus, subterranean clover stunt virus etc. These viruses belong to the Luteovirus group characterized by the presence of spherical particles about 25 nm in diameter. Electron microscopic observation of leaf-dip preparations or ultrathin sections from CSV-A infected plants did not reveal any virus-like particles. This observation suggests that CSV does not belong to the Luteovirus group and may belong to a new virus group.

### Literature cited

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