TOMATO SPOTTED WILT VIRUS ISOLATED FROM PEANUT IN THAILAND

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Abstract

Two viruses belonging to the tomato spotted wilt virus group were isolated from peanut showing chlorosis and necrosis on young leaves. These viruses infected a wide range of host plants, and had a thermal inactivation point of 40–45°C for 10 min, dilution end point of 10–100 times, and longevity in vitro of about 2 hr at 20°C. In ultrathin sections, infected cells contained spherical particles 80–100 nm in diameter with an envelope and wrapped by endoplasmic reticulum.

1. Introduction

During surveys of virus diseases of peanut, many peanut plants showing severe symptoms including chlorosis and necrosis of young leaves were observed in Kalasin and Roi-et, north-eastern Thailand. The causal agents of these diseases were found to contain two types of viruses in inoculation tests to some differential host plants. This paper describes some properties of these viruses.

2. Materials and methods

The viruses (P79-11 and P79-36) were isolated from peanut plants showing chlorosis and necrosis on young leaves that were collected in Kalasin and Roi-et, north-eastern Thailand in 1979.

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

In host range tests, the viruses were inoculated mechanically to 32 plant species in nine families. Symptomless plants were assayed by back-inoculation to *Chenopodium amaranticolor* using sap extracted from inoculated leaves 7–10 days after inoculation and from newly emerged leaves about 21 days after inoculation. All plant species were tested at least twice in different seasons.

Stability in crude sap was determined using crude juice prepared by macerating 1 g diseased leaves together with 5 ml of 0.05 M phosphate buffer, pH 7.2, containing 10 mM DIECA and 1 mM L-cysteine. *C. amaranticolor* was used as test plant.

Dip preparations were prepared by the direct negative staining method.

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In ultrathin sections, small pieces of diseased peanut leaves were fixed in 3% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.0, and post-fixed in 1% osmium tetroxide in the same buffer. Then, after dehydration in a alcohol series, the tissues were embedded in Spurr resin. Ultrathin sections were cut with glass knives and stained with uranyl acetate and lead citrate. These preparations were examined under a Hitachi Model H-500 or H-300 electron microscope.

3. Results

1) The virus

Diseased peanut plants showed chlorotic spots or chlorosis on young leaves, and some plants also showed necrosis of leaves or buds. Inoculation tests to some differential hosts proved that these symptoms were caused by two viruses (P79-11 and P79-36) with very different host reactions.

2) Host range

P79-11. The virus infected 16 plant species in six families among 32 species in nine families inoculated with the extract of *Nicotiana glutinosa* infected with the virus (Table 1).

Tetragonia expansa showed systemic chlorotic spots or chlorotic rings.

Arachis hypogaea showed systemic chlorotic spots and bud necrosis.

Datura stramonium showed systemic necrotic spots, vein-clearing and vein necrosis.

Lycopersicon esculentum showed systemic chlorotic spots and necrotic rings.

Nicotiana clevelandii showed yellowing and rolling of young leaves, and plant growth decreased more severely than in healthy plant.

N. glutinosa showed systemic yellowing and rolling of young leaves.

Spinacia oleracea did not develop symptoms, but back-inoculation tests indicated that the plants were systemically infected with the virus.

Chenopodium amaranticolor, C. quinoa, Vigna sesquipedalis, Nicotiana tabacum, and *Petunia hybrida* showed chlorotic or necrotic local lesions on inoculated leaves.

Vigna radiata, Cucumis sativus, and *Zinnia elegans* did not show any symptoms, but back-inoculation tests indicated that the inoculated leaves of these plants contained the virus.

Other 16 plant species were not susceptible to the virus.

P79-36. The virus infected 21 plant species in 7 families among 32 species in 9 families inoculated with the extract of diseased leaves of *Nicotiana clevelandii* (Table 1).

Tetragonia expansa showed systemic chlorotic or necrotic spots.

Arachis hypogaea showed systemic severe symptoms including vein-clearing, chlorotic spots, mosaic and bud necrosis (Fig. 1).

Nicotiana clevelandii showed systemic yellowing and leaf curling.

Spinacia oleracea did not develop symptoms, but back-inoculation tests indicated that this plant was systemically infected with the virus.

Chenopodium amaranticolor, C. quinoa, Gomphrena globosa, Dolichos lablab, Phaseolus vulgaris, Vigna radiata, V. sequipedalis, V. unguiculata, Nicotiana glutinosa, and *Petunia hybrida* showed chlorotic or necrotic local lesions. Since the local lesions on *G. globosa* were distinct and characteristic, this plant appears to be very useful for

differential hosts (Fig. 2).

Table 1. Host range of tomato spotted wilt virus

Plant	P79-11		P79-36	
	IL	NIL	IL	NIL
Chenopodium amaranticolor	L	-	L	-
C. quinoa	L	-	L	-
Spinacia oleracea	1	s	1	s
Gomphrena globosa	-	-	L	-
Tetragonia expansa	1	M	L	CS, NS
Brassica rapa	-	-	-	-
Arachis hypogaea	1	CS, NS	1	VC, CS, N
Astragalus sinicus	-	-	-	-
Canavalia ensiformis	-	-	-	-
Dolichos lablab	-	-	L	-
Glycine max	-	-	-	-
Lathyrus odoratus	-	-	-	-
Lupinus luteus	-	-	-	-
Phaseolus angularis	-	-	1	-
P. vulgaris	-	-	L	-
Pisum sativum	-	-	1	-
Trifolium pratense	-	-	-	-
T. repens	-	-	-	-
Vicia faba	-	-	-	-
Vigna mungo	-	-	1	-
V. radiata	1	-	L	-
V. sesquipedalis	L	-	L	-
V. unguiculata	L	-	L	-
Datura stramonium	1	NS, VC, VN	L	NR
Lycopersicon esculentum	1	CS, NR	-	-
Nicotiana clevelandii	1	Y, St	L	Y, St
N. glutinosa	1	M, St	L	-
N. tabacum	L	-	L	-
Petunia hybrida	L	-	L	-
Sesamum indicum	-	-	-	-
Cucumis sativus	1	-	1	-
Zinnia elegans	1	-	1	-

IL: inoculated leaves, NIL: non-inoculated leaves, L: local lesion, M: mosaic, CS: chlorotic spot, VC: vein clearing, N: necrosis, VN: vein necrosis, NR: necrotic ring, Y: yellowing, St: stunting, l, s: symptomless infection in inoculated or non-inoculated leaves, respectively, -: no infection.

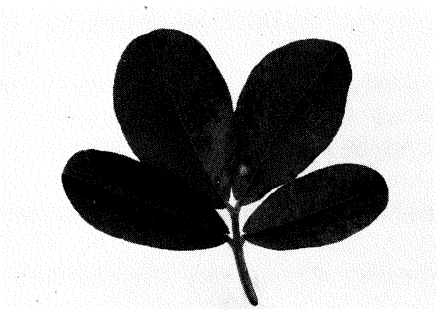

Fig. 1. Chlorotic spots and mosaic symptoms on peanut infected with tomato spotted wilt virus (P79-36).

Fig. 2. Local lesions on inoculated leaves of *Gomphrena globosa* with tomato spotted wilt virus (P79-36).

Phaseolus angularis, Pisum sativum, Vigna mungo, Cucumis sativus, Zinnia elegans did not develop symptoms, but back-inoculation tests indicated that the inoculated leaves of these plants contained the virus.

Other 11 plant species were not susceptible to the virus.

3) Stability in crude sap

Stability in crude sap of P79-11 and P79-36 was determined using the sap from diseased leaves of *N. glutinosa* and *N. clevelandii,* respectively.

P79-11 was infective after heating at 40°C for 10 min, but not at 45°C, dilution to 10^{-1}, but not 10^{-2}, and storage at 20°C for 2 hr, but not 6 hr.

P79-36 was infective after heating at 40°C for 10 min, but not 45°C, dilution to 10^{-1}, but not 10^{-2}, and storage at 20°C for 1 hr, but not 2 hr.

4) Electron microscopy

P79-11. In ultrathin sections of diseased peanut leaves, virus-like particles 80–100 nm in diameter with endoplasmic reticulum were observed in the cytoplasm (Fig. 3). These particles were not observed in the cytoplasm of healthy peanut leaves.

P79-36. The preparations were made by the direct negative staining method using diseased peanut leaves fixed previously with 3% glutaraldehyde. Virus-like particles 80–100 nm in diameter with envelopes and wrapped by endoplasmic reticulum were observed (Fig. 4).

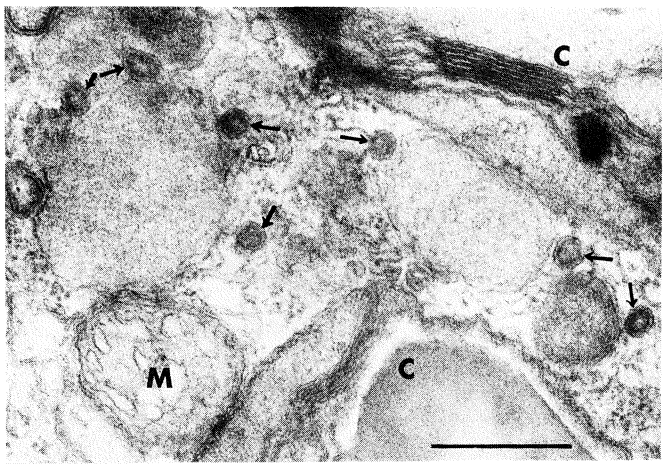

Fig. 3. Virus-like particles (arrows) in ultrathin sections of peanut infected with tomato spotted wilt virus (P79-11). C: chloroplast, M: mitochondria, Bar = 500 nm.

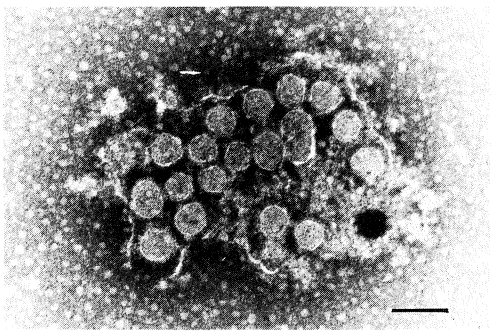

Fig. 4. Virus-like particels in direct negative staining preparation of fixed peanut leaf infected with tomato spotted wilt virus (P79-36). Bar = 200nm.

4. Discussion

These viruses were identified as tomato spotted wilt virus (TSWV) based on host range, symptomatology, stability in crude sap, and particle morphology.

TSWV occurs worldwide on many crops, for example, tomato, tobacco, chilli, peanut, dahlia, pineapple, amaryllis, etc. suggesting a very wide host range (1). TSWV is also severely impairing the production of these crops especially in tropical countries, due to the presence of high populations of thrips, vector of TSWV, in these countries.

In Thailand, the occurrence of TSWV was shown first. While TSWV was detected only in two locations, the virus may be spreading more widely because TSWV has a very wide host range and also the population of thrips was very high.

Many isolates giving symptoms differing in severity had been reported (2). Isolate P79-11 is similar to the type strain of TSWV in its host reactions. Isolate P79-36 is

very different from these viruses.

References cited

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