PEANUT MOTTLE VIRUS OCCURRING ON PEANUT IN THAILAND

Fumiyoshi FUKUMOTO[1], Pornpod THONGMEEARKOM[2], Mitsuro IWAKI[3], Duangchi CHOOPANYA[4], Nonglak SARINDU[4], Nualchan DEEMA[4], and Tsuneo TSUCHIZAKI[1]

Abstract

A virus isolated from a peanut plant showing chlorotic spot symptoms that was collected at Kalasin, North East Thailand in 1979, was identified as peanut mottle virus based on host range, symptomatology, aphid transmissibility, particle morphology, serological relationships, etc. The virus was transmitted by sap inoculation and by aphid, but not through seed of peanut from Japan. The virus infected 14 plant species in four families and infected systemically peanut, pea, broad bean, soybean, etc. with visible symptoms. It also produced local lesions on inoculated leaves of Top Crop bean. Thermal inactivation point, dilution end point and longevity in vitro of the virus were 55-60°C (10 min), 10^{-4}-10^{-5}, and 7-14 days (20°C), respectively. The virus consisted of filamentous flexuous particles, 700-725 nm in length. The virus was purified from infected pea plants, and the yield of purified virus was about 0.76 mg from 100 g plant materials. The virus reacted positively with antiserum to peanut mottle virus from Japan.

1. Introduction

During surveys of virus diseases of peanut plants in Thailand, mottle disease was observed in wide areas.

The inoculation tests of the diseased samples to differential host plants showed that these plants were infected with two viruses. One of the viruses produced necrotic local lesions on the inoculated leaves of Top Crop bean, and the other virus produced chlorotic local lesions on the inoculated leaves of *Chenopodium amaranticolor*.

This paper describes some properties of the first virus that was isolated by propagation from one local lesion on inoculated leaves of Top Crop bean and was identified as peanut mottle virus.

2. Materials and methods

1) Virus The virus was isolated from naturally infected peanut plants showing chlorotic spots collected at Kalasin, North East Thailand in 1979.

All the test plants were grown in a glasshouse. Sap inoculation was carried out by rubbing the leaf surface previously dusted with Carborundum (600 mesh) with a cotton piece soaked in inoculum.

---

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

2) Host range and symptoms Host range of the virus was investigated by mechanical inoculation using diseased leaves of Kintoki bean. Symptomless plants were assayed by back inoculation to Kintoki bean using sap extracted from inoculated leaves 7–10 days after inoculation and from newly emerged leaves about 28 days after inoculation. All plant species were tested at least twice in different seasons.

3) Transmission Aphid transmission tests from diseased peanut to healthy peanut were carried out using aphid, *Aphis craccivora,* reared on healthy broad bean. Aphids allowed to fast for 2 hr in a glass beaker were transferred to diseased peanut for an acquisition access of 10 min. After the acquisition access, groups of 10 aphids were transferred to healthy peanut for an inoculation access of 2 hr. Then these aphids were transferred again to new healthy peanut for a second inoculation access of 24 hr. These aphids were removed by spraying with insecticides after the second inoculation access was completed.

Seed transmission tests were carried out using seeds from infected peanut plants grown in a glasshouse.

4) Stability in crude sap Thermal inactivation point (TIP), dilution end point (DEP) and longevity in vitro (LIV) of these viruses were determined using crude juice of diseased leaves of Kintoki bean. Top Crop bean was used as assay host plant. Crude juice was prepared by grinding 6 g of diseased leaves with 30 ml of 0.05 M phosphate buffer, pH 7.0, and squeezing through cheesecloth. In the test of LIV, after storage of each periods the crude juice was diluted tenfold with the same buffer as that used for the inoculation.

5) Electron microscopy Dip preparations were obtained by grinding a small piece of diseased leaf of Kintoki bean in 2–3 drops of 2% ammonium molybdate, and mounting the extract on a carbon-stabilized Formvar-coated grid.

6) Purification and serology The virus was purified from infected pea plants. Infected leaves and stems of infected pea plants were homogenized with 1.5 volumes of 0.5 M potassium phosphate buffer, pH 8.0, including 0.1% 2-mercaptoethanol (2-ME) and 10 mM sodium ethylenediaminetetraacetic acid (EDTA). Sap was expressed through cheesecloth and processed by clarification with chloroform and carbon tetrachloride, precipitation by polyethylene glycol (#6000), differential centrifugation, sucrose density gradient centrifugation (Fig. 1). Purified virus was used in the serological tests.

Serological relationships of the virus with some potyviruses were analyzed by ring tests using purified virus.

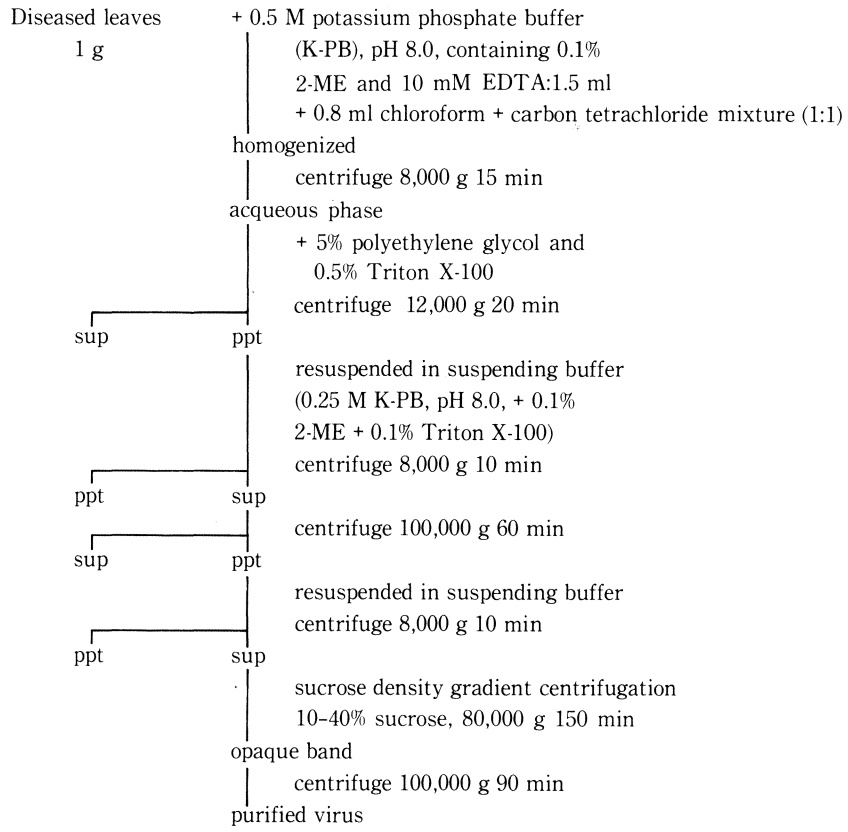

Fig. 1. Purification procedure of peanut mottle virus.

3. Results

1) Host range and symptoms

The virus infected 14 plant species in four families among the 28 species in nine families tested (Table 1).

Arachis hypogaea showed systemic mild mottle symptoms.

Glycine max showed systemically vein-clearing and mottle. Subsequently these symptoms became indistinct.

Phaseolus vulgaris cv. Kintoki showed chlorotic local lesions on inoculated leaves and systemic leaf curling and necrosis.

Pisum sativum, Vicia faba, Nicotiana clevelandii, and *Sesamum indicum* showed systemic chlorotic spots or mosaic.

Gomphrena globosa, Vigna mungo, V. sesquipedalis, V. unguiculata, and *Petunia hybrida* did not show any symptoms. However, these plants were found to be infected systemically with the virus by back inoculation to Kintoki bean.

Phaseolus vulgaris cv. Top Crop showed necrotic local lesions on inoculated leaves (Fig. 2), but was not infected systemically.

Table 1. Host range of peanut mottle virus

Plant	Symptoms	
	IL	NIL
Chenopodium amaranticolor	-	-
C. quinoa	-	-
Gomphrena globosa	1	-
Tetragonia expansa	-	-
Brassica rapa	-	-
Arachis hypogaea	1	Mo
Glycine max	1	Mo
Lathyrus odoratus	1	-
Lupinus luteus	-	-
Phaseolus vulgaris 'Kintoki'	L	Mal, M
" 'Top Crop'	L	-
Pisum sativum	1	CS, Mo
Trifolium pratense	-	-
T. repens	-	-
Vicia faba	1	CS, Mo
Vigna mungo	1	s
V. radiata	1	-
V. sesquipedalis	1	s
V. unguiculata	1	s
Datura stramonium	-	-
Lycopersicon esculentum	-	-
Nicotiana clevelandii	1	CS, M
N. glutinosa	-	-
N. tabacum	-	-
Petunia hybrida	1	s
Sesamum indicum	1	Mo
Cucumis sativus	-	-
Zinnia elegans	-	-

IL: inoculated leaves, NIL: non-inoculated leaves, L: local lesion, Mo: mottle, Mal: leaf rolling, CS: chlorotic spot, M: mosaic, 1 and s: symptomless infection on inoculated leaves and non-inoculated leaves, respectively, -: no infection.

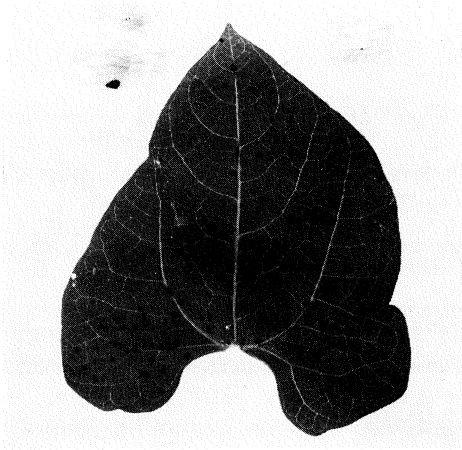

Fig. 2. Local lesions on inoculated leaf of Top Crop bean produced by peanut mottle virus.

Vigna radiata and *Lathyrus odoratus* did not show any symptoms. However, back-inoculation to Kintoki bean indicated that the inoculated leaves of these plants contained the virus.

The virus did not infect the other 13 plant species.

2) Transmission

(1) Aphid transmission. *Aphis craccivora* transmitted the virus from diseased peanut to healthy peanut in the first inoculation access (7/8, 2/8), but not in the second one (0/8, 0/8), suggesting that the virus was transmitted in a non-persistent manner (Table 2).
(2) Seed transmission. The virus was not transmitted through 302 of the seeds collected from diseased peanut plants.

Table 2. Transmission tests of peanut mottle virus by *Aphis craccivora*

Experiment	First inoculation access [b)]	Second inoculation access [c)]
1	7/8 [a)]	0/8
2	2/8	0/8

a): number of infected plants/number of inoculated plants,
b): access period = 2 hr,
c): access period = 24 hr,
Preacquisition fasting period = 2 hr,
Acquisition access period = 10 min,
Number of aphids per test plant = 10 insects.

3) Stability in crude juice

Thermal inactivation point, dilution end point and longevity in vitro of the virus ranged between 55 and 60°C for 10 min, 10^{-4} and 10^{-5}, and 7 and 14 days at 20°C, respectively.

4) Electron microscopy

Dip preparations stained negatively with 2% ammonium molybdate from infected Kintoki bean leaves showed filamentous flexuous particles, most of which were 700-800 nm in length with a peak of 700-725 nm.

5) Purification and serology

The virus was purified from infected pea plants. A single opaque band typical of a virus-containing zone was observed in sucrose density gradients. Yield of the purified virus was about 0.76 mg from 100 g plant materials.

The virus reacted positively with antiserum to peanut mottle virus from Japan (PnMV-PN) (1) (homologous titer, 1 : 128 in ring test) until a dilution of 1 : 256 in ring test (Table 3). On the other hand, the virus showed distant or no relationships with other potyviruses, namely, peanut chlorotic ring mottle virus (homologous titer, 1 :

Table 3. Reaction of peanut mottle virus to antisera to four potyviruses in ring test

Antisera	Dilution of antisera							
	4	8	16	32	64	128	256	512
PnMV-PN	+	+	+	+	+	+	+	-
PCRMV	+	+	-	-	-	-	-	-
BlCMV	+	-	-	-	-	-	-	-
BCMV	-	-	-	-	-	-	-	-
SMV	-	-	-	-	-	-	-	-

Antigen: purified peanut mottle virus, OD_{260} = 0.2,
PnMV-PN: peanut mottle virus from Japan, PCRMV: peanut chlorotic ring mottle virus, BlCMV: blackeye cowpea mosaic virus, BCMV: bean common mosaic virus, SMV: soybean mosaic virus.

256), blackeye cowpea mosaic virus (1 : 128), bean common mosaic virus (1 : 64), and soybean mosaic virus (1 : 128) (Table 3).

4. Discussion

As described above, the virus that produced local lesions on inoculated leaves of Top Crop bean was shown to have similar properties to those of peanut mottle virus (PnMV) reported by Kuhn (2) and Inouye (1). Moreover, the virus showed close serological relationships with PnMV from Japan (1). Based on these results, the virus was identified as peanut mottle virus.

The virus was detected from only two samples among approximately 150 samples collected, suggesting that it was not widespread in Thailand.

PnMV is transmitted through seed of peanut at a rate of a few percent (1, 2) and this seed-borne virus is very important as a virus source in the field. In this experiment, the virus was not transmitted through seeds of peanut. This negative result may be related to differences in cultivars of peanut or glasshouse conditions, especially temperature.

Literature cited

1. Inouye, T. (1969). Peanut mottle virus from peanut and pea. Nogaku Kenkyu 52: 159-164.
2. Kuhn, C.W. (1965). Symptomatology, host range, and effect on yield of a transmitted peanut virus. Phytopathology 55 : 880-884.