PEANUT CHLOROTIC RING MOTTLE VIRUS OCCURRING ON PEANUT IN THAILAND

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Abstract

A virus was isolated from peanut plants showing distinct mottle, yellow ring mottle, chlorotic spot, chlorotic ring symptoms. The virus which consists of filamentous flexuous particles 730-750 nm in length was transmitted by sap inoculation and by aphid, but not through seeds of peanut. The virus infected 16 plant species in six families, and produced chlorotic local lesions on inoculated leaves of *Chenopodium amaranticolor*. Thermal inactivation point, dilution end point and longevity in vitro of the virus were 55-60°C (10 min), 10^{-4}-10^{-5}, and 14-21 days (20°C), respectively. Pinwheel and bundle type inclusion bodies typical of those of potyviruses were observed in the cytoplasm of the infected cells. The virus showed very distant serological relationships with peanut mottle virus from Japan and Thailand. On the other hand, the virus showed close serological relationships with blackeye cowpea mosaic virus, bean common mosaic virus and soybean mosaic virus. Since the virus differs in host range and serology from other potyviruses reported from peanut, the virus was designated as peanut chlorotic ring mottle virus.

1. Introduction

Peanut plants showing mottle, yellow ring mottle, chlorotic spot, chlorotic ring, etc. were observed over wide areas in the fields of Thailand.

A virus with filamentous flexuous particles and producing chlorotic local lesions on inoculated leaves of *Chenopodium amaranticolor*, was isolated from most of these peanut plants.

The virus has similar properties to those of peanut mottle virus (PnMV) except for the reactions on local host plants. Therefore, the virus had been previously designated as ring-type strain of PnMV. However, since the virus showed very distant serological relationships with PnMV, it was suggested that the virus was different from PnMV, and it was thus renamed peanut chlorotic ring mottle virus.

This paper describes properties and serological relationships of the virus designated as peanut chlorotic ring mottle virus.

2. Materials and methods

1) Virus The virus was isolated from naturally infected peanut plants showing distinct mottle symptoms that were collected at Kalasin, North East Thailand in 1979.

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

2) Host range and symptoms Host range of the virus was determined by mechanical inoculation using diseased leaves of Kintoki bean. 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 28 days after inoculation. All the 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 were allowed to fast for 2 hr in a glass beaker before 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 plants for a second inoculation access of 24 hr. These aphids were removed by spraying insecticide after the second inoculation access.

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 the virus were determined using crude sap of diseased leaves of Kintoki bean. *C. amaranticolor* was used as assay host plant. Crude sap was prepared by grinding 6 g of diseased leaves with 30 ml of 0.05 M phosphate buffer, pH 7.0, squeezed that through cheesecloth. In the test of LIV, after storage of each periods the crude juice was diluted in tenfold with the same buffer and assayed.

5) Electron microscopy Dip preparations were prepared by grinding a small piece of diseased leaf of Kintoki bean in 2-3 drops of 2% phosphotungstic acid, pH 6.5, and mounting the extract on a carbon-stabilized, Formvar-coated grid.

For ultrathin sections, a small piece of diseased leaves was fixed in 2% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.0, for 30 min, then post-fixed in 1% osmium tetroxide in the same buffer. These samples were dehydrated by an ethanol series and mounted in Spurr resin. Ultrathin sections were cut using glass knives. These sections were stained with uranyl acetate and lead citrate and examined under a Hitachi Model H-500 or H-300 electron microscope.

6) Purification and serology The virus was purified from infected leaves of Kintoki bean following the procedure adopted for peanut mottle virus. Infected leaves were homogenized with 1.5 volumes of 0.5 M potassium phosphate buffer, pH 8.0, including 0.1% 2-mercaptoethanol and 10 mM sodium ethylenediaminetetraacetic acid. Sap was expressed through cheesecloth and processed by polyethylene glycol (#6000), differential centrifugation, sucrose density gradient centrifugation.

Rabbits were immunized by two intravenous injections of purified virus and two intramuscular injections of the virus mixed with Freund's complete adjuvant. Antiserum was obtained from blood collected 10 days after the final injection. The serological relationships of the virus with some potyviruses were analyzed by ring tests and tube precipitin tests using purified virus or sap from infected leaves.

3. Results

1) Host range and symptoms

The virus infected 16 plant species in six families among the 27 species in nine families tested (Table 1).

Arachis hypogaea showed systemically chlorotic ring, symptoms which developed to mottle symptoms (Fig. 1).

Glycine max showed distinct mosaic symptoms systemically.

Phaseolus vulgaris cv. Kintoki showed leaf curling and mosaic symptoms.

Vicia faba, Vigna mungo, V. sesquipedalis, Nicotiana clevelandii, Petunia hybrida, Sesamum indicum did not develop symptoms. However, back-inoculation tests to *Chenopodium amaranticolor* indicated that these plants were infected systemically with the virus.

Chenopodium amaranticolor (Fig. 2), *C. quinoa* and *Tetragonia expansa* showed chlorotic local lesions on the inoculated leaves. These plants were not infected systemically with the virus.

Gomphrena globosa, Lathyrus odoratus, and *Vigana unguiculata* did not show

Table 1. Host range of peanut chlorotic ring mottle virus

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

IL: inoculated leaves, NIL: non-inoculated leaves, L: local lesion, Mo: mottle, M: mosaic, VN: vein necrosis, Mal: leaf rolling, 1 and s: symptomless infection in inoculated and non-inoculated leaves, respectively, -: no infection.

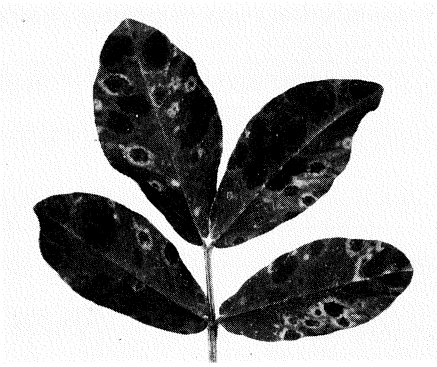

Fig. 1. Chlorotic ring and mottle symptoms on peanut caused by peanut chlorotic ring mottle virus.

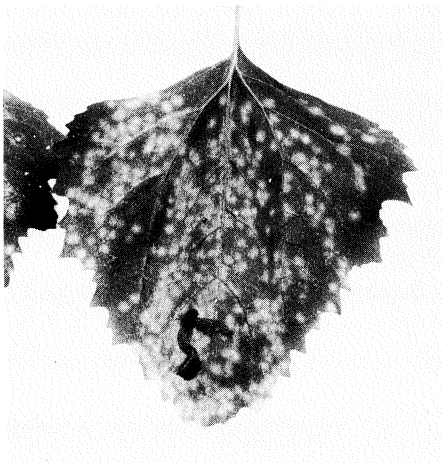

Fig. 2. Local lesions on inoculated leaf of *Chenopodium amaranticolor* caused by peanut chlorotic ring mottle virus.

symptoms, but back-inoculation to *C. amaranticolor* indicated that the inoculated leaves of these plants contained the virus.

Other 12 plant species were not infected with the virus.

2) Transmission

Aphid transmission. *Aphis craccivora* transmitted the virus in a first inoculation access (6/6, 7/8), but not in a second inoculation access (0/6, 0/8), suggesting that the virus was transmitted in a non-persistent manner (Table 2).

Seed transmission. The virus was not transmitted through the 308 seeds collected from diseased peanuts in the glasshouse.

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

Experiment	First inoculation access[a)]	Second inoculation access[b)]
1	6/6[c)]	0/6
2	7/8	0/8

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

3) Stability in crude sap

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 14 and 21 days at 20°C, respectively.

4) Electron microscopy

Dip preparations negatively stained with 2% potassium phosphotungstate, pH 6.5, from infected peanut leaves showed filamentous flexuous particles, most of which were 730-750 nm in length (Fig. 3).

In ultrathin sections of diseased peanut leaves, pinwheel and bundle type inclusion bodies typical of those of potyviruses were observed in the cytoplasm of the infected cells (Fig. 4, 5).

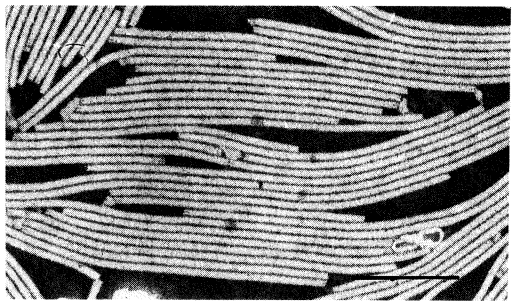

Fig. 3. Purified virus particles of peanut chlorotic ring mottle virus. Bar = 200 nm.

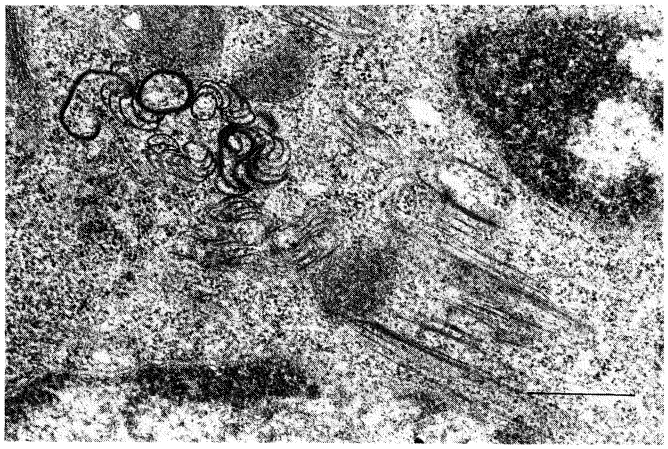

Fig. 4. Pinwheel and bundle type inclusion bodies in ultrathin sections of peanut leaf infected with peanut chlorotic ring mottle virus. Bar = 500 nm.

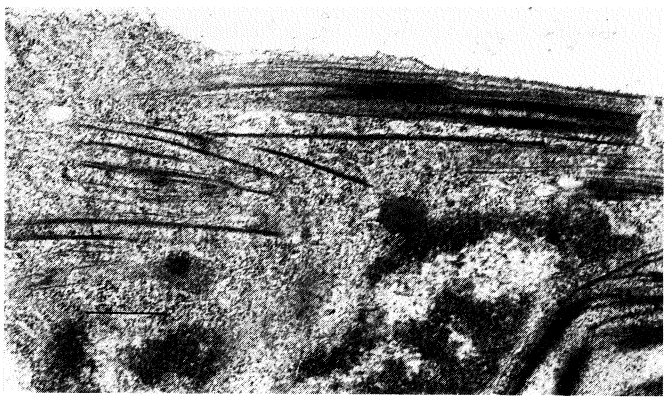

Fig. 5. Bundle type inclusion bodies in ultrathin sections of peanut leaf infected with peanut chlorotic ring mottle virus. Bar = 500 nm.

5) Purification

The virus was purified from infected Kintoki bean leaves. A single opaque band typical of a virus-containing zone was observed in sucrose density gradients. The yield of purified virus was 10-20 mg from 100 g of infected leaves of Kintoki bean.

6) Serology

The virus showed close serological relationships with blackeye cowpea mosaic virus (homologous titer, 1 : 128), bean common mosaic virus (1 : 64), and soybean mosaic virus (1 : 128), but distant serological relationships with peanut mottle virus from Japan (1 : 256) (Table 3, 4).

Table 3. Reactions of peanut chlorotic ring mottle virus to antisera to four potyviruses in ring tests

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

Purified virus of PCRMV, OD_{260} = 0.2,
PnMV-PN: PnMV from Japan,
BlCMV: blackeye cowpea mosaic virus,
BCMV: bean common mosaic virus,
SMV: soybean mosaic virus.

Table 4. Reactions of three viruses to antiserum to peanut chlorotic ring mottle virus in tube precipitin tests

Antigen	Dilution of antiserum								
	4	8	16	32	64	128	256	512	1024
PnMV-PN	+	-	-	-	-	-	-	-	-
PnMV-T	+	±	-	-	-	-	-	-	-
PCRMV	+++	+++	+++	+++	++	++	+	+	+
Juice of healthy bean	-	-	-	-	-	-	-	-	-

Antigen was prepared by grinding diseased leaves with 0.25 M potassium phosphate buffer, pH 8.0 and centrifuged at 8,000 g 10 min.
PnMV-PN: PnMV from Japan, PnMV-T: PnMV from Thailand.

4. Discussion

Peanut chlorotic ring mottle virus (PCRMV) was characterized as a potyvirus based on transmission mode, virus particle and inclusion body morphology, and serology.

Many potyviruses, namely peanut mottle virus (PnMV) (3), groundnut mosaic virus (GMV) (5), groundnut eyespot virus (GEV) (2), peanut green mosaic virus (PGMV) (4), virus causing peanut mild mottle (VPMM) (6) and peanut stripe virus (PStV) (1), were reported from peanut plants throughout the world.

PCRMV differs from PnMV and PEV in host range and serology, and PCRMV produced local lesions on inoculated leaves of *C. amaranticolor* which PnMV did not infect. And also, PCRMV failed to infect Top Crop bean while PnMV produced local lesions.

PCRMV is similar to GMV in the host reaction and particle morphology, but serological relationships have not been determined.

PGMV, VPMM and PStV produce local lesions on inoculated leaves of *C. amaranticolor* as in the case of PCRMV. However, PGMV and VPMM differ from

PCRMV in their host range and serological relationships with other potyviruses. PStV seemed to be slightly different from PCRMV in host reactions and rate of seed transmissibility.

Although PCRMV must be compared in detail with these potyviruses, PCRMV seemed to be a new virus on peanut.

In this experiment, PCRMV was not transmitted through seeds of peanut from Japan. This negative result may be attributed to differences in cultivars of peanut or glasshouse conditions especially temperature.

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