

# Peanut Chlorotic Ring Mottle, a Potyvirus Occurring Widely on Southeast Asian Countries

By FUMIYOSHI FUKUMOTO,\*<sup>1a</sup> PORNPOD THONGMEEARKOM,\*<sup>2</sup>  
MITSURO IWAKI,\*<sup>3</sup> DUANGCHAI CHOOPANYA,\*<sup>4</sup> TSUNEO TSUCHIZAKI,\*<sup>1</sup>  
NORIO IIZUKA,\*<sup>5</sup> NONGLAK SARINDU,\*<sup>4</sup> NUALCHAN DEEMA,\*<sup>4</sup>  
CHING ANG ONG\*<sup>6</sup> and NASIR SALEH\*<sup>7</sup>

\*<sup>1</sup> National Agriculture Research Center (Yatabe, Ibaraki, 305 Japan)

\*<sup>2</sup> Department of Agriculture (Bangkhen, Bangkok, 10900, Thailand)

\*<sup>3</sup> National Institute of Agro-Environmental Sciences (Yatabe, Ibaraki, 305 Japan)

\*<sup>4</sup> Department of Agriculture (Bangkhen, Bangkok, 10900, Thailand)

\*<sup>5</sup> Hokkaido National Agricultural Experimental Station (Toyohira, Sapporo, 004 Japan)

\*<sup>6</sup> Malaysian Agriculture Research and Development Institute (Serdang, Selangor, Malaysia)

\*<sup>7</sup> Bogor Research Institute for Food Crops (Bogor, Indonesia)

## Introduction

Mottle diseases including yellow ring mottle, chlorotic ring, chlorotic spot, etc. of peanut occur very widely in Thailand, Malaysia and Indonesia.

Two viruses consisting of filamentous flexuous particles were isolated from diseased plants. The first virus was identified as a peanut mottle virus (PnMV) on the basis of its host range, symptomatology and serological relationships. The second one producing chlorotic local lesions on inoculated leaves of *Chenopodium amaranticolor* was isolated from most of the diseased peanut plants. The virus showed very distant serological relationship with PnMV, showing that it is different from PnMV. It was reported that the same virus

was isolated from soybean plants in Malaysia.<sup>9)</sup>

We previously reported the second virus as the ring type strain of PnMV from symptoms and particle morphology,<sup>7)</sup> but based on further experiments the virus was identified as a new potyvirus, and designated as a peanut chlorotic ring mottle virus (PCRMV).

This paper describes some properties of PCRMV and serological relationships with other potyviruses pathogenic to legumes.

## Materials and methods

**Virus source** A virus designated as PCRMV was obtained from naturally infected peanut plants collected at Kalasin, Northeast Thailand, in 1979. After three successive single-lesion transfers on *C. amaranticolor*, the isolate was propagated in Kintoki bean.

All test plants were grown in a glasshouse. Sap inoculation was carried out by rubbing

---

Present address:

\*<sup>1a</sup> National Institute of Agro-Environmental Sciences (Yatabe, Ibaraki, 305 Japan)

\*<sup>2</sup> Thai Monsanto (Bangkok, Thailand)

Carborundum (600 mesh)-dusted leaf surface with a piece of cotton soaked in inoculum. The inoculum was prepared by grinding diseased leaves in 0.05 M phosphate buffer (P.B.), pH 7.0, containing 0.02% KCN with a mortar and a pestle.

**Host range and symptoms** Host range of the virus was investigated by mechanical inoculation using crude extracts from diseased leaves of Kintoki bean. Symptomless plants were assayed by back inoculation to *C. amaranticolor*, 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 three times in different seasons.

**Transmission** Aphid transmission tests were carried out. *Aphis craccivora*, reared on a healthy broad bean, was allowed to fast for 2 hr in glass beakers and then transferred to diseased peanut plants for acquisition access of 1–5 min. After that 10 aphids were transferred to a healthy peanut for inoculation access of 2 hr. Then they were transferred again to a new healthy peanut for secondary inoculation access of 24 hr.

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

**Stability in crude sap** Thermal inactivation point (TIP), dilution end point, and longevity in vitro (LIV) of the virus were determined using crude extracts of diseased leaves of Kintoki bean. *C. amaranticolor* was used as assay plants. Crude extracts were prepared by grinding 6 g of diseased leaves with 30 ml of 0.05 M P.B., pH 7.0, and squeezing through cheesecloth. Crude extracts used for TIP and LIV tests were then diluted tenfold with the same buffer and assayed.

**Electron microscopy** Dip preparations were prepared by grinding a small piece of diseased leaf of Kintoki bean in 2–3 drops of 2% potassium phosphotungstic acid, pH 6.5, and mounting the extract on a carbon-stabilized, Formvar-coated grid. The length of PCRMV particles was estimated using tobacco mosaic virus particles (300 nm) as a standard

of length.

To prepare ultrathin sections small pieces of diseased leaves were fixed in 2% glutaraldehyde in 0.1 M Na-P.B., pH 7.0, for 30 min, and then post-fixed in 1% osmium tetroxide in the same buffer for 4 hr. They were dehydrated by ethanol series and embedded in Spurr resin. Ultrathin sections were cut with glass knives. These sections were stained with uranyl acetate and lead citrate, and examined under the Hitachi Model H-500 or H-300 electron microscope.

**Purification** PCRMV was purified from infected Kintoki bean plants. The infected leaves were homogenized with 1.5 volumes (w/v) of 0.5 M K-P.B., pH 8.0, including 0.1% 2-mercaptoethanol (2-ME), 10 mM sodium ethylenediamine tetraacetate (EDTA) and 0.8 volume (w/v) of a mixture of chloroform and carbon tetrachloride (1 : 1). The preparation was centrifuged for 15 min at 8,000 g and the supernatant fluid was recovered. Polyethylene glycol (MW 6,000) and Triton X-100 were added to the supernatant fluid to give a final concentration of 5% and 0.5%, respectively. After stirring for 0.5–1 hr, the mixture was centrifuged for 20 min at 12,000 g, and the sediment was resuspended in suspending buffer (0.25 M K-P.B., pH 8.0, including 0.1% 2-ME and 0.1% Triton X-100). After one cycle of differential centrifugation, the supernatant was centrifuged in 10–40% sucrose density gradients in suspending buffer for 150 min at 80,000 g. The zone containing virus particles was collected, and diluted with equal volume of the buffer. The virus was concentrated by two cycles of ultracentrifugation, and a final pellet was resuspended in 10 mM Na-P.B., pH 7.0. The virus yield was calculated spectrophotometrically using an extinction coefficient,  $E_{260\text{ nm}}^{0.1\%} = 2.7.4^1$

**Serology** Rabbits were immunized by injecting twice intravenously with purified PCRMV and twice intramuscularly with the virus mixed with Freund's complete adjuvant. Total virus injected was 8.5 mg to a rabbit. Antiserum obtained from blood collected 10 days after final injection reacted on purified

virus in ring interface precipitin tests (ring tests) at a dilution of 256.

Blackeye cowpea mosaic virus (B1CMV), bean common mosaic virus (BCMV), soybean mosaic virus (SMV) or PnMV-PN from Japan,<sup>8)</sup> and PnMV-T isolate from Thailand were used to compare their serological relationships. PnMV-PN was provided by T. Inouye. Antisera to four isolates of PnMV (EA type, *Cassia*, *Voandzeia* and *Phaseolus lunatus*) and peanut stripe virus (PStV) were provided by K. R. Bock and J. W. Demski, respectively.

The serological relationships of the virus with some potyviruses were analyzed by ring tests and agar gel double diffusion tests using purified virus. The latter tests were conducted in 0.85% agarose with 0.85% NaCl, 0.4% lithium 3,5-diiodosalicylate, and 0.1% sodium azide. In some of agar gel double diffusion tests, the sharp non-specific line was observed near the wells of purified virus.

## Results

Host range and symptoms Out of 27

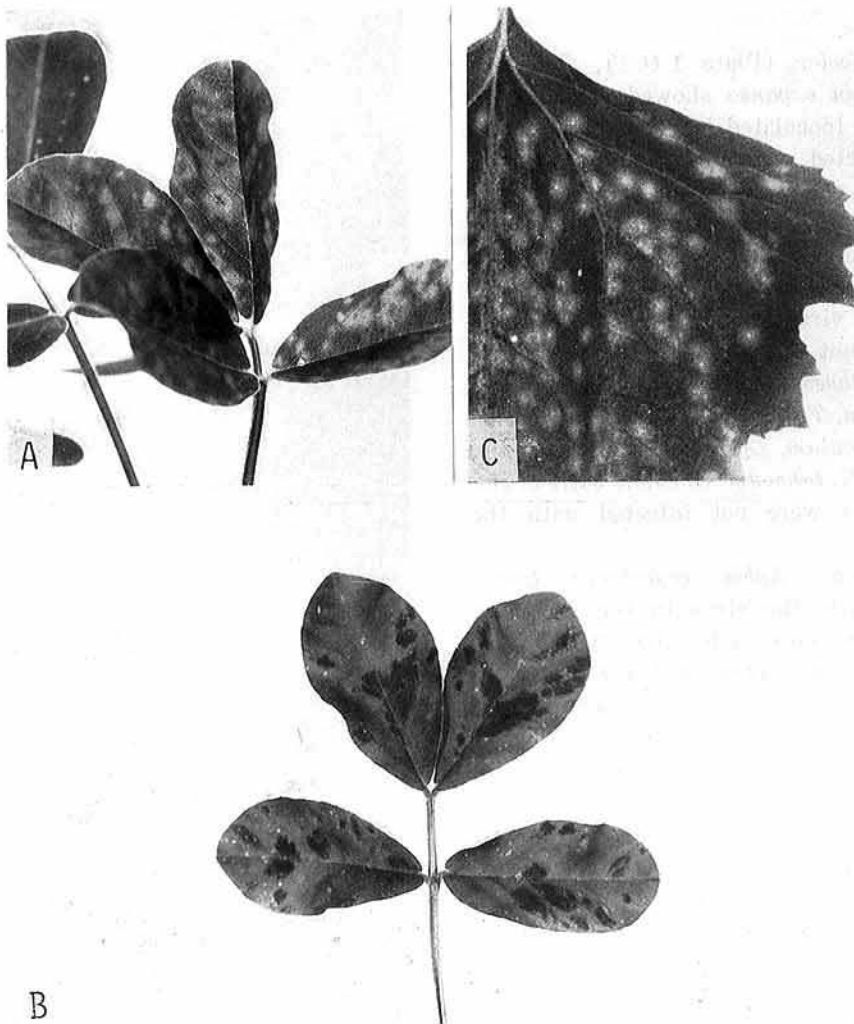


Plate 1. Symptoms caused by peanut chlorotic ring mottle virus  
 (A) Ringspots on peanut at an early stage of infection  
 (B) Chlorotic mottle symptoms on peanut  
 (C) Chlorotic local lesions on *Chenopodium amaranticolor*

species of 9 families tested 16 plant species of 6 families were susceptible to PCRMV. *Arachis hypogaea* showed systemically distinct mottle, yellow ring mottle, chlorotic spot, and chlorotic ring (Plate 1 (A) and (B)). *Glycine max* (cv. Shirotsurunoko and Okuharawase) showed distinct mosaic symptoms systemically. *Phaseolus vulgaris* cv. Kintoki showed leaf curling and mosaic. *Vicia faba*, *Vigna mungo*, *V. sesquipedaris*, *Nicotiana clevelandii*, *Petunia hybrida*, *Sesamum indicum* did not develop symptoms, but back-inoculation tests to *C. amaranticolor* indicated that these plants were infected systemically with the virus.

*C. amaranticolor* (Plate 1 (C)), *C. quinoa* and *Tetragonia expansa* showed chlorotic local lesions on inoculated leaves. These plants were not infected systemically with the virus.

*Gomphrena globosa*, *Lathyrus odoratus* and *Vigna unguiculata* did not show symptoms, but back-inoculation tests to *C. amaranticolor* indicated that inoculated leaves of these plants contained the virus.

Other 12 plant species, *Brassica rapa*, *Lupinus luteus*, *Phaseolus vulgaris* (cv. Top Crop), *Pisum sativum*, *Trifolium pratense*, *T. repens*, *Datura stramonium*, *Lycopersicon esculentum*, *N. glutinosa*, *N. tabacum*, *Cucumis sativus* and *Zinnia elegans* were not infected with the virus.

**Transmission** *Aphis craccivora* transmitted efficiently the virus in the first inoculation access (6/6, 7/8), but not in the second inoculation access (0/6, 0/8) showing that the transmission was a non-persistent type. The virus was not transmitted through 308 seeds collected from diseased peanut in a glasshouse.

**Stability in crude juice** Thermal inactivation point, dilution end point and longevity in vitro of the virus were 55–65°C for 10 min,  $10^{-4}$ – $10^{-5}$ , and 14–21 days at 20°C, respectively.

**Electron microscopy** Electron micrographs of dip preparations taken from infected bean leaves showed filamentous flexuous particles, and 90% of the 138 particles examined were 730–750 nm in length (Plate 2 (A)).

In ultrathin sections of diseased peanut leaves, pinwheel or bundle type inclusion bodies typical to potyviruses were observed in cytoplasm (Plate 2 (B)).

**Purification** After sucrose density gradi-

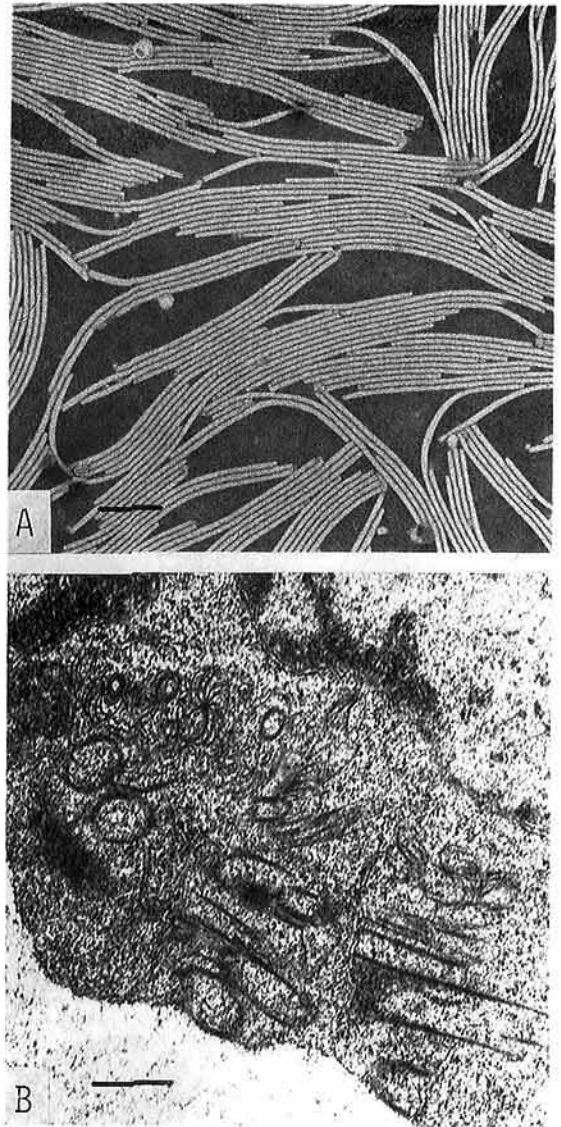


Plate 2. Electron micrograph of particles and inclusion bodies of peanut chlorotic ring mottle virus (Bar=200 nm)

- (A) Purified virus preparation stained with 1% potassium phosphotungstic acid  
 (B) Pinwheel and bundle type inclusion bodies observed in a thin section

ent centrifugation, a single opalescent virus band was visible in gradient tubes. The yield of purified virus was 13–22 mg from 100 g of infected leaves of Kintoki bean. However, the yield decreased to 2–7 mg when EDTA was removed from extraction buffer. The UV absorption spectrum of the purified PCRMV had a maximum at 261 nm, and a minimum at 247–248 nm. The ratio of  $A_{260}/A_{280}$  was 1.22.

**Serology** PCRMV reacted on antisera to BICMV (homologous titre, 1 : 128) diluted up to 1 : 128, BCMV (homologous titre, 1 : 64) diluted up to 1 : 32, SMV (homologous titre, 1 : 128) diluted up to 1 : 64, and PnMV-PN (homologous titre, 1 : 128) diluted up to 1 : 8 in ring tests. In agar gel double diffu-

sion tests, PCRMV reacted on antisera to BICMV, BCMV and SMV with spur, but did not on antiserum to PnMV-PN (Table 1).

In another test, PCRMV, BICMV, BCMV and SMV reacted on antiserum to PCRMV diluted up to 256 in ring tests, and also these viruses formed precipitin line against antiserum to PCRMV in agar gel double diffusion tests. Only SMV showed spur (Plate 3(A)). However, PnMV-PN reacted on antiserum to PCRMV diluted up to 32 in ring tests, and also showed weaker precipitin line with spur against antiserum to PCRMV in agar gel double diffusion tests. On the other hand, antiserum to PnMV-PN showed no reaction or weak reaction on PCRMV, BICMV, BCMV

**Table 1. Serological reaction of the isolates of peanut chlorotic ring mottle virus to antisera of other potyviruses in ring tests and agar gel double diffusion tests**

Antisera <sup>a)</sup>	Antigen <sup>a)</sup>		
	PCRMV	PnMV-T	PnMV-PN
BICMV	128 <sup>b)</sup> (+) <sup>c)</sup>	4 (+)	0 (+)
BCMV	32 (+)	0 —	0 (+)
SMV	64 (+)	0 —	0 —

a) Viral isolates and antisera used: PCRMV: peanut chlorotic ring mottle virus. PnMV-T and PnMV-PN are a type strain of peanut mottle virus (PnMV) in Thailand and an isolate of PnMV in Japan, respectively. BICMV: blackeye cowpea mosaic virus, BCMV: bean common mosaic virus, SMV: soybean mosaic virus.

b) The figure represents reciprocal titres of antisera in ring tests. The concentration of the purified viruses is  $OD_{260} = 0.2$ . The homologous titres of BICMV, BCMV and SMV antisera in ring tests were 1 : 128, 1 : 64, 1 : 128, respectively.

c) The signs represent reaction in agar gel double diffusion tests. + = Reaction of homologous or identity. — = No reaction. (+) = Reaction forming spur.

**Table 2. Serological reaction of the isolates of peanut chlorotic ring mottle virus and other potyviruses to homologous and heterologous antisera in ring tests and agar gel double diffusion tests**

Antisera <sup>a)</sup>	Antigens <sup>a)</sup>					
	PCRMV	PnMV-T	PnMV-PN	BICMV	BCMV	SMV
PCRMV	256 <sup>b)</sup> + <sup>c)</sup>	8 (+)	32 (+)	128 +	256 +	128 (+)
PnMV-PN	8 —	256 (+)	128 +	0 —	0 —	32 —

Legends in this table: See Table 1.

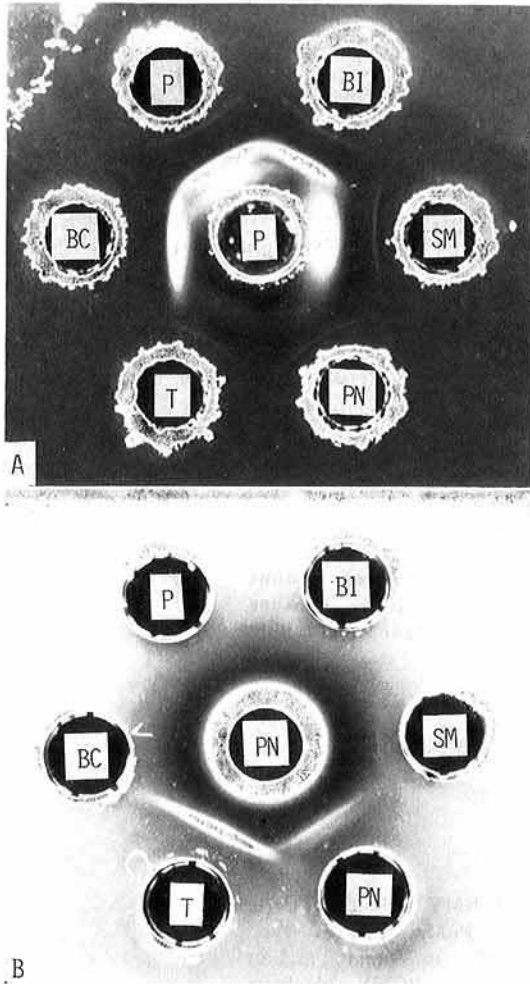


Plate 3. Agar gel double diffusion tests with peanut chlorotic ring mottle virus (PCRMV), blackeye cowpea mosaic virus (BICMV), bean common mosaic virus (BCMV), soybean mosaic virus (SMV) and peanut mottle virus (PnMV-PN and PnMV-T) in agar gel diffusion plates containing lithium 3,5-diiodosalicylate

Central wells in (A) and (B) are filled with antiserum to PCRMV and PnMV-PN, respectively. The peripheral wells are filled with purified preparations of PCRMV (P), BICMV (BI), BCMV (BC), SMV (SM), PnMV-PN (PN) and PnMV-T (T).

and SMV in ring tests and did not react on these viruses in agar gel diffusion tests (Plate 3 (B), Table 2).

From these results, PCRMV showed dis-

tant serological relationship with PnMV-PN (type strain), and close relationship with BICMV, BCMV and SMV although PCRMV is a little different because these viruses showed spur in agar gel. Also, an isolate of PCRMV from Indonesia reacted positively in agar gel and in immunosorbent electron microscopy on antiserum to PStV.

No reaction was observed when purified PCRMV was tested against antisera to peanut green mosaic virus (PGMV) and 4 isolates of PnMV from Africa<sup>2)</sup> in ring tests and and agar gel double diffusion tests.

### Discussion

On the basis of host range, transmission, in vitro properties, virus particle and inclusion body morphology, and serology, the virus causing chlorotic ring, followed by mottle symptoms, on peanut plants in Thailand, Malaysia and Indonesia, was identified as a potyvirus. The virus had a similar host range with PnMV-T from Thailand and PnMV-PN from Japan,<sup>8)</sup> except reactions on Top Crop bean and *C. amaranticolor*, but showed distant relationship in serological tests with PnMV-PN. Therefore, the virus was designated as a peanut chlorotic ring mottle virus (PCRMV).

Four viruses related to PnMV occurring in East Africa produced local lesions on *C. amaranticolor* and *C. quinoa* like PCRMV. But, antisera to these viruses did not react on PCRMV, showing that these viruses are distinct from PCRMV.

Other four potyviruses, groundnut eyespot virus (GEV),<sup>6)</sup> peanut green mosaic virus (PGMV),<sup>13)</sup> peanut stripe virus (PStV)<sup>5)</sup> and the virus causing peanut mild mottle disease (PMMV)<sup>18)</sup> were reported from naturally infected peanut.

PCRMV differs from GEV and PGMV in symptomatology on peanut and host range. GEV infects *Lycopersicon esculentum*, which PCRMV does not infect, but fails to infect *C. amaranticolor*. PGMV resembles PCRMV on reactions to *C. amaranticolor*, *C. quinoa* and Top Crop bean, but PGMV does not in-

fect *Vicia faba*, *Vigna mungo* and soybean. And, PCRMV is unrelated to PGMV serologically.

PMMV and PCRMV cause chlorotic spots and ringspots on peanut, and infect *C. amaranticolor* and *C. quinoa*, but not Top Crop bean, which is characteristic local lesion host of PnMV.<sup>3)</sup> Both viruses are distantly related to PnMV serologically. But PMMV does not infect *Vigna sesquipedalis*, *V. unguiculata* and *Petunia hybrida*, which PCRMV infects. PMMV showed no serological relationship with SMV but PCRMV is closely related to SMV. Also, it was reported that the purification of PMMV was very difficult, and the virus yield was low. But it was very easy to purify PCRMV and its yield was higher as compared to other potyviruses from peanut. Because of these several differences, it seems that PMMV is distinct from PCRMV.

Recently, PStV was reported as a new potyvirus infected peanut.<sup>5)</sup> Because of close serological relationship between PStV and PMMV, they were suspected strains of one virus even though some differences were found.<sup>5)</sup> PCRMV is similar to PStV in host range and in serological relationship with BICMV and SMV and also an isolate of PCRMV reacted positively on antiserum to PStV. On the contrary, PStV does not cause chlorotic rings on peanut like PCRMV. And PCRMV shows distant serological relationship with PnMV, though PStV shows no reaction on antiserum to PnMV. Additionally, seed transmission rate of PStV in peanut is much higher as compared to PnMV reported previously.<sup>1,8,10,12)</sup> PCRMV is not transmitted through seeds of Japanese peanut cultivars. On the other hand, further comparative studies between PCRMV and PStV are necessary to confirm the relationship between the two viruses.

The Indonesian isolate of PnMV which is seed-born in peanut (3%),<sup>12)</sup> is very similar to PCRMV on the host range, symptoms<sup>12)</sup> and serological relationship. Groundnut mosaic virus (GMV) isolated in Malaysia<sup>15)</sup> also is very similar to PCRMV on the host range and symptoms, though serological relationship

is not tested.

As described above, PCRMV occurs very widely on peanut in Thailand, and also it was isolated from peanut collected in Indonesia and Malaysia.<sup>9)</sup> The wide occurrence of Indonesian isolate of PnMV in Indonesia and GMV in Malaysia was reported so far. Thus, it is quite likely that PCRMV prevails widely on peanuts in Southeast Asian countries.

Our results of reciprocal agar gel double diffusion tests with antisera to PCRMV, BICMV, BCMV and SMV showed that PCRMV was very closely related to BICMV, BCMV and SMV, and PStV, too. The results of previous work showed that in agar gel double diffusion tests close serological relationship existed between BICMV and BCMV,<sup>16)</sup> and SMV was distantly related to BICMV and BCMV.<sup>16,17)</sup> The serological relationships among potyviruses are, in general, highly complex. Further studies of serological nature are needed with PCRMV, BICMV, BCMV and SMV.

## Summary

A virus consisting of filamentous flexuous rods, 730–750 nm long, was isolated from peanut plants (*Arachis hypogaea*) showing distinct mottle, yellow ring mottle, chlorotic spot, chlorotic ring symptoms in Thailand, Malaysia and Indonesia. The virus was readily transmissible by sap inoculation, and by aphid in a non-persistent manner, but not through seeds of peanut. The virus infected 16 plant species of six families, and produced chlorotic local lesions on inoculated leaves of *Chenopodium amaranticolor*. Longevity of the virus in vitro was 14–21 days at 20°C, a thermal inactivation point 55–60°C, and a dilution end point 10<sup>-4</sup>–10<sup>-5</sup>. Pinwheel or bundle type inclusion bodies were observed in cytoplasm of infected leaf tissue. The virus showed very distant serological relationship with peanut mottle virus from Thailand and Japan, and positive reaction to black-eye cowpea mosaic virus, bean common mosaic virus and soybean mosaic virus. Since the virus differs in host range, symptomatology,

transmission characteristics and serology from other potyviruses reported occurring on peanut, the virus was designated as a peanut chlorotic ring mottle virus.

### Acknowledgement

We thank to Dr. K. R. Bock, East Africa Agriculture and Forestry Research Organization, Kenya, and Dr. J. W. Demski, University of Georgia, U.S.A. for providing antisera to PnMV and PSTV, respectively, and Dr. T. Inouye, University of Osaka Prefecture, Japan for providing PnMV.

### References

- 1) Adams, D. B. & Kuhn, C. W.: Seed transmission of peanut mottle virus in peanuts. *Phytopathology*, **67**, 1126-1129 (1977).
- 2) Bock, K. R., Guthrie, E. J. & Gina Meredith.: Viruses occurring in East Africa that are related to peanut mottle virus. *Ann. appl. Biol.*, **89**, 423-428 (1978).
- 3) Bock, K. R. & Kuhn, C. W.: *C.M.I./A.A.B. Descriptions of Plant Viruses* No. 141 (1975).
- 4) Choi, J. K., Maeda, T. & Wakimoto, S.: An improved method for purification of turnip mosaic virus. *Ann. Phytopathol. Soc. Jpn.*, **43**, 440-448 (1977).
- 5) Demski, J. W. et al.: Peanut stripe virus—a new seed-borne potyvirus from China infecting groundnut (*Arachis hypogaea*). *Ann. appl. Biol.*, **105**, 495-501 (1984).
- 6) Dubern, J. & Dollet, M.: Groundnut eye-spot virus, a new member of the potyvirus group. *Ann. appl. Biol.*, **96**, 193-200 (1980).
- 7) Fukumoto, F. et al.: Peanut mottle and tomato spotted with viruses of peanut (*Arachis hypogaea*) in Thailand. *Ann. Phytopathol. Soc. Jpn.*, **49**, 81 (1983) [Abstr. in Japanese].
- 8) Inouye, T.: Peanut mottle virus from peanut and pea. *Nogaku Kenkyu*, **52**, 159-164 (1969) [In Japanese].
- 9) Iwaki, M. et al.: Viruses isolated from soybean and peanut plants in Malaysia. *Ann. Phytopathol. Soc. Jpn.*, **50**, 441 (1984) [Abstr. in Japanese].
- 10) Kuhn, C. W.: Symptomatology, host range, and effect on yield of a seed-transmitted peanut virus. *Phytopathology*, **55**, 880-884 (1965).
- 11) Reddy, D. V. R. et al.: The occurrence of peanut mottle virus in India. *Plant Dis. Repr.*, **62**, 978-982 (1978).
- 12) Roechan, M. M. et al.: Virus diseases of legume plants in Indonesia 4. Peanut mottle virus. *Contribution Centr. Res. Inst. Agr. Bogor*, **46**, 1-11 (1978).
- 13) Sreenivasula, P. et al.: Peanut green mosaic virus—a member of the potato virus Y group infecting groundnut (*Arachis hypogaea*) in India. *Ann. appl. Biol.*, **98**, 255-260 (1981).
- 14) Taiwa, M. A. et al.: Partial characterization and grouping of isolates of blackeye cowpea mosaic and cowpea aphidborne mosaic viruses. *Phytopathology*, **72**, 590-596 (1982).
- 15) Ting, W. P., Geh, S. L. & Lim, Y. C.: Studies on groundnut mosaic virus of *Arachis hypogaea* L. in West Malaysia. *Expl. Agri.*, **8**, 355-368 (1972).
- 16) Tsuchizaki, T. et al.: Blackeye cowpea mosaic virus from asparagus bean (*Vigna sesquipedalis*) in Thailand and Malaysia, and their relationships to a Japanese isolate. *Ann. Phytopathol. Soc. Jpn.*, **50**, 461-468 (1984).
- 17) Tsuchizaki, T., Thongmeearkom, P. & Iwaki, M.: Soybean mosaic virus isolated from soybeans in Thailand. *JARQ*, **15**, 279-285 (1982).
- 18) Xu, Z. et al.: A virus causing peanut mild mottle in Hubei Province, China. *Plant Disease*, **67**, 1029-1032 (1983).

(Received for publication, February 1, 1986)