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Virus Diseases Occurring on Some Vegetable Crops in West Malaysia

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Introduction

In 1984, the authors were given an opportunity to observe the occurrence and distribution of virus diseases on vegetables in West Malaysia under the project "Promotion of Vegetable Production in the Tropics". Many virus diseases of chilli (hot pepper), sweet pepper, tomato, eggplant, pumpkin and cucumber plants were observed, and preliminary tests of causal viruses were carried out at MARDI in Cameron Highlands. Since the period of the study at MARDI was limited, leaf samples were brought to Vegetable and Ornamental Crops Research Station in Japan for identification of causal viruses. In the present report, properties of the viruses isolated from some vegetables in West Malaysia will be described.

Materials and methods

Mechanical inoculation was carried out using a glass spatula dipped in sap of infected leaves on Carborundum dusted fully expanded cotyledons or leaves of test plants. The sap was prepared by grinding infected leaves in a mortar with 0.1 M phosphate buffer, pH 7.0, containing 0.1% thioglycolic acid. *Myzus persicae* and *Aphis gossypii* were used for a test of aphid transmission. The aphids starved for 2 hr were fed on infected plants for 10 min, and then 5 aphids were transferred to each healthy seedling. The aphids were killed after remaining on the test plant for 2 hr. The purified virus and crude leaf extract were negatively stained with 2% phosphotungstic acid, pH 7.0, after fixation with 10% formalin or 1% OsO_4 vapor, and examined under electron microscopy. Two kinds of immunodiffusion media were used for serological tests. The one consisted of 0.8% Noble agar with 0.85% sodium chloride in 0.01 M phosphate buffer, pH 7.0, and was used for tests of small spherical virus particles. The other consisted of 0.8% Noble agar with 1% sodium azide and 0.5% sodium lauryl sulfate (SDS) in distilled water, and was used for tests of filamentous virus particles.

Results and discussion

1) *Chilli (Capsicum annuum and C. frutescens)*

Chilli is a lowland crop in West Malaysia and is reported to be grown on about 910 ha in 1979. The occurrence of virus disease has become a major constraint on production. In the present survey, more than 80% of the cultivated crops were found to be infected with mosaic disease in the field. Insect

vectors, thrips and aphids, were observed on chilli in many fields, and larvae and adults of thrips were often observed on chilli plants showing severe mosaic or top necrosis symptom. No white flies, however, were observed.

Eighteen samples of virus infected chilli plants were collected in Bertam Valley of Cameron Highlands, Subang Jaya in Selangor, and Air Keroh, Pontian and Ulu Belitong in Johor. The plants showed mosaic, yellow mosaic, top necrosis, or mixture of these symptoms. For preliminary classification, sap of each sample was inoculated mechanically to diagnostic test plants, i.e., *Capsicum annuum* (hot pepper), *Lycopersicon esculentum, Petunia hybrida, Nicotiana tabacum* (Xanthi nc), *N. glutinosa, Vicia faba, Vigna sesquipedalis* and *Chenopodium quinoa*. From 9 samples among 18, virus was transmitted to test plants, but the rest 9 samples could not infect any of test plants. Five types of symptom on *C. annuum* were tentatively distinguished and identification of causal virus was carried out for them. The results are as follows.

(1) Mosaic symptoms with chlorotic vein banding

Symptoms on the original chilli plants were in most cases mosaic with chlorotic vein banding or vein yellow (Plate 1). A virus was isolated from the leaves of *N. glutinosa* showing systemically vein yellow symptoms, and it induced clear mosaic symptoms and sometimes leaf distortion on *C. annuum*.

Host range Systemic symptoms were produced by the virus in the following species: *N. tabacum* (Xanthi nc, White Burley and Bright Yellow), *N. megalosiphon, N. clevelandii* and *P. hybrida*. Tobacco (White Burley) produced systemic veinal mottle symptom. On *N. clevelandii*, the virus induced systemic mosaic symptom 5 days after inoculation. The following species failed to be infected: *Datura stramonium, C. amaranticolor, C. quinoa, V. faba, Pisum sativum, Cucurbita pepo* and *Cucumis sativus*.

Aphid transmission Infected *C. annuum* and 6 healthy seedlings of *C. annuum* were used as a source plant and a test plant, respectively. The virus was transmitted to all of test plants both by *M. persicae* and *A. gossypii*.

Virus particles Filamentous virus particles were readily observed in leaf dip-preparations from chilli, and their length ranged from 700 to 750 nm. In purified preparations, particles with normal length and also fragmented particles were contained (Plate 2). In ultrathin sections of *N. clevelandii* leaves infected with the virus, cytoplasmic pinwheel or bundle type inclusions were observed. In SDS-agar gel double diffusion tests, crude sap of infected leaves did not react with potato virus Y (PVY)[2] or pepper veinal mottle virus.[1]

These properties of the virus were very similar to those of chilli veinal mottle virus (CVMV) reported by Ong et al.[6]

(2) Top necrosis symptoms

Symptoms on the field-infected chilli plants were top necrosis, necrotic spots on leaves and necrotic streaks on stems. Two mechanically transmissible

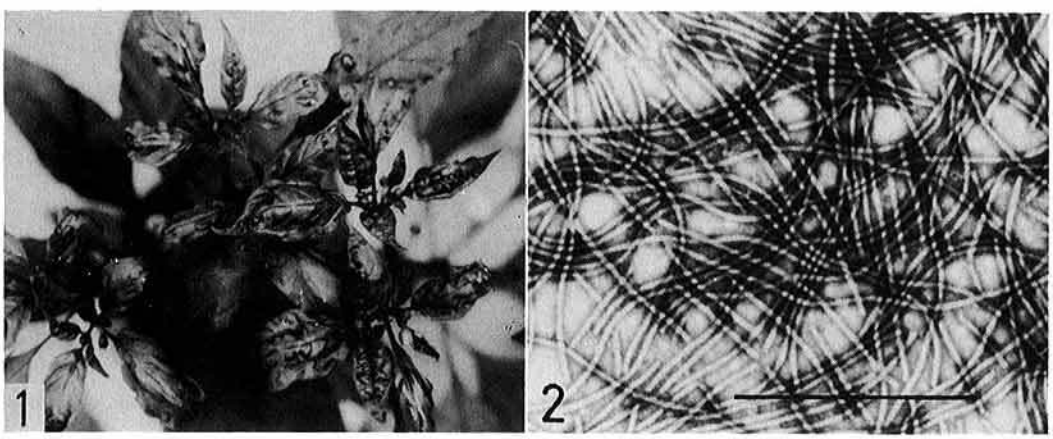


Plate 1. Severe mosaic symptoms in hot pepper caused by infection of chilli veinal mottle virus
Plate 2. Electron micrograph of purified chilli veinal mottle virus particles (Bar represents 500 nm)

viruses were detected from these diseased chilli plants. The one was CVMV. Another virus having large spherical particles was isolated from necrotic local lesions on the inoculated leaves of *P. hybrida*. On *C. annuum*, the spherical virus induced characteristic top necrosis symptoms and necrotic streaks on leaves, stems or fruit (Plate 3). Some properties of the spherical virus were investigated.

Host range Systemic symptoms were produced in the following species: *N. tabacum* (Xanthi nc), *N. glutinosa, N. megalosiphon, N. sylvestris, N. clevelandii, D. stramonium, L. esculentum, Spinacia oleracea, Zinnia elegans* and *Tetragonia expansa*. The symptoms observed were pronounced necrosis in many of the susceptible plants, especially those plants belonging to Solanaceae. The following species produced local lesions on inoculated leaves without subsequent systemic infection: *V. faba, P. sativum, Beta vulgaris, C. amaranticolor* and *C. quinoa*.

Virus particles When fixed with OsO_4 vapor before negative staining with 2% phosphotungstic acid (pH 7.0), leaf-dip preparations from diseased plants contained large spherical particles, 70–100 nm in diameter, surrounded by an outer layer structure (Plate 4). In ultrathin sections of the infected leaf cells of tomato, small clusters of spherical virus particles of 70–90 nm in diameter enclosed in vesicles were frequently observed in cytoplasm.

Since these results obtained in this experiment coincided with the results of Kobatake et al,[5] the spherical virus was identified as tomato spotted wilt virus (TSWV).

(3) Mild mosaic symptoms

The field-infected chilli plants generally showed mild mosaic symptom and did not show any necrotic symptoms. These diseased plants were revealed to be infected with 2 viruses on the basis of inoculation tests to diagnostic test plants and electron microscopy. The one was CVMV. Another virus having rod-shaped particles, 300 nm in the length was isolated from necrotic local lesions on the inoculated leaves of *N. glutinosa*. The virus induced systemic mild mosaic symptoms on *C. annuum* 7 days after inoculation. Some properties of the rod-shaped virus were investigated.

Host range The virus produced systemic mosaic on *N. tabacum* (Samsun), *L. esculentum, Z. elegans* and local lesions on inoculated leaves of *P. hybrida, N. sylvestris, N. tabacum* (Bright Yellow) and *C. amaranticolor*.

Virus particles Electron micrographs of sap from infected plants showed rod-shaped particles with a modal length of 300 nm × 18 nm.

From these results, the virus was identified as a tomato strain of tobacco mosaic virus (TMV-L).

(4) Severe yellow mosaic symptoms

Symptoms on the chilli plants in the field were systemic severe yellow mosaic. These diseased

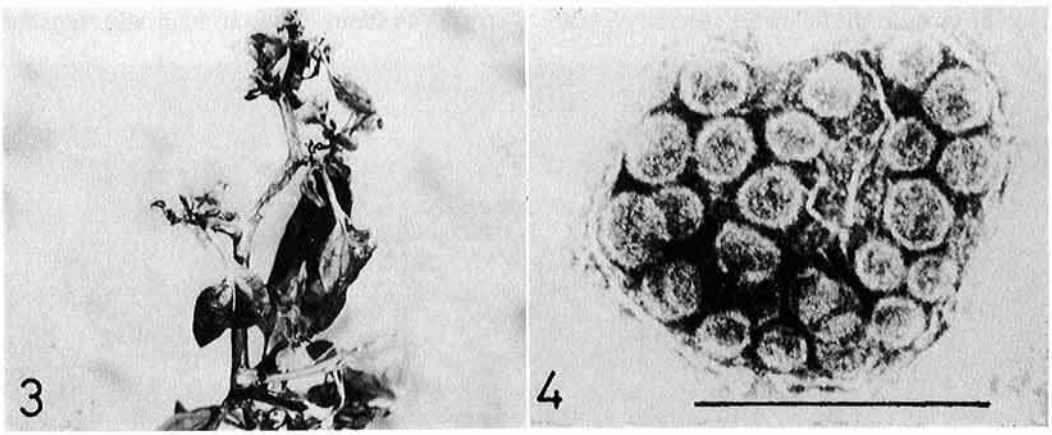


Plate 3. Top necrosis symptom and necrotic streaks on stem in hot pepper caused by infection of tomato spotted wilt virus

Plate 4. Electron micrograph of tomato spotted wilt virus particles inside the membrane in leaf dip preparation (Bar represents 500 nm)

plants were found to be infected with 2 viruses on the basis of inoculation test to diagnostic test plants and electron microscopy. The one was CVMV. Another virus was spherical particles, and it induced systemic mosaic symptoms on *C. annuum*.

Host range The virus produced systemic mosaic on *N. glutinosa, P. hybrida, L. esculentum, D. stramonium* and *C. sativus*. Furthermore, the virus formed necrotic local lesions on the inoculated leaves of *V. faba, P. sativum, V. sesquipedalis* and *Sesamum indicum*. Many spherical particles about 30 nm in diameter were observed in leaf dip-preparations from infected leaves.

Serological reaction The extracts of systemically infected tobacco leaves reacted positively with the antiserum against Y strain of cucumber mosaic virus (CMV-Y) in the immunodiffusion test and the precipitin lines to the virus and CMV-Y fused each other.

These results lead to the conclusion that the virus is an ordinary strain of CMV (CMV-O).

(5) Severe mosaic symptoms with leaf distortion

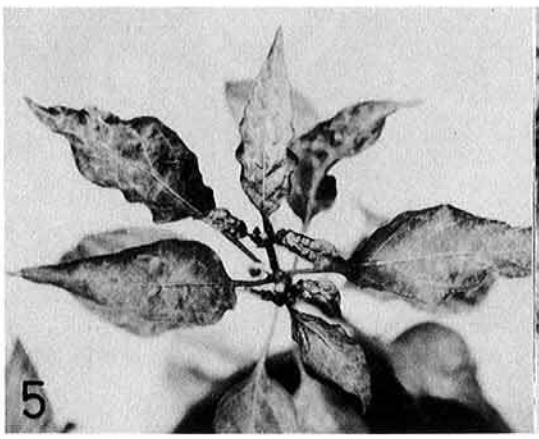
Symptoms on the field-grown chilli plants were systemic severe mosaic and dwarfing of plants. These chilli plants were found to be infected doubly with 2 viruses on the basis of inoculation tests to diagnostic test plants. The one was CVMV. Another was isolated from necrotic local lesion on the inoculated leaves of *N. glutinosa*, and induced clear mosaic symptoms and sometimes chlorotic vein banding on *C. annuum* (Plate 5).

Host range The host range of the virus resembled that of CMV-O above described. On *C. pepo* and *C. sativus*, however, the virus induced characteristic necrotic local lesions on the inoculated leaves without subsequent systemic infection. The spherical particles about 30 nm in diameter were observed in leaf dip-preparations and in ultrathin sections from infected leaves (Plate 6).

Aphid transmission Infected *C. annuum* and 6 healthy seedlings of *C. annuum* were used as a source plant and a test plant, respectively. The virus was transmitted to 3 of 6 plants and 5 of 6 by *M. persicae* and *A. gossypii*, respectively.

Serological reaction Antiserum against CMV-Y positively reacted with the virus, and a faint spur developed between the precipitin lines to the virus and CMV-Y. When the antiserum against pepper strain of CMV (CMV-P) was used,[4] a spur developed between the precipitin lines to the virus and CMV-P. Antiserum against the virus was prepared using the same procedure as described previously[3] and its titer was 1/512 in the ring interface precipitin test. This antiserum reacted positively both with CMV-Y and CMV-P, and spurs developed between precipitin lines to the virus and CMV-Y or CMV-P.

Because these properties of the virus differed from those of CMV-Y or CMV-P, the virus may be distinguished from these 2 strains of CMV, and was tentatively designated as a chilli strain of CMV (CMV-C).



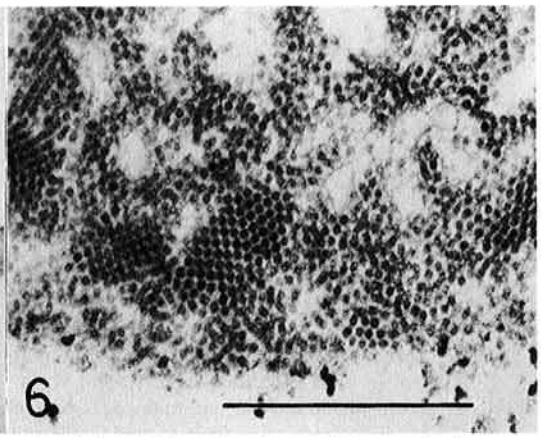


Plate 5. Mosaic symptom in hot pepper caused by infection of the chilli strain of cucumber mosaic virus
Plate 6. The aggregate of the chilli strain of cucumber mosaic virus particles in the cytoplasm of infected tobacco leaf cell (Bar represents 500 nm)

So far, the isolation of CVMV, TMV and TSWV from diseased chilli was reported in West Malaysia. From the present study, 2 strains of CMV, CMV-O and CMV-C, were also found to infect chilli plants. Although PVY was reported to be the most common cause of severe chilli losses in Sri Lanka,[6] we could not isolate the virus from any samples of infected chilli.

2) Sweet pepper

Another survey on the virus diseases occurring on sweet pepper was made at Bertam Valley and

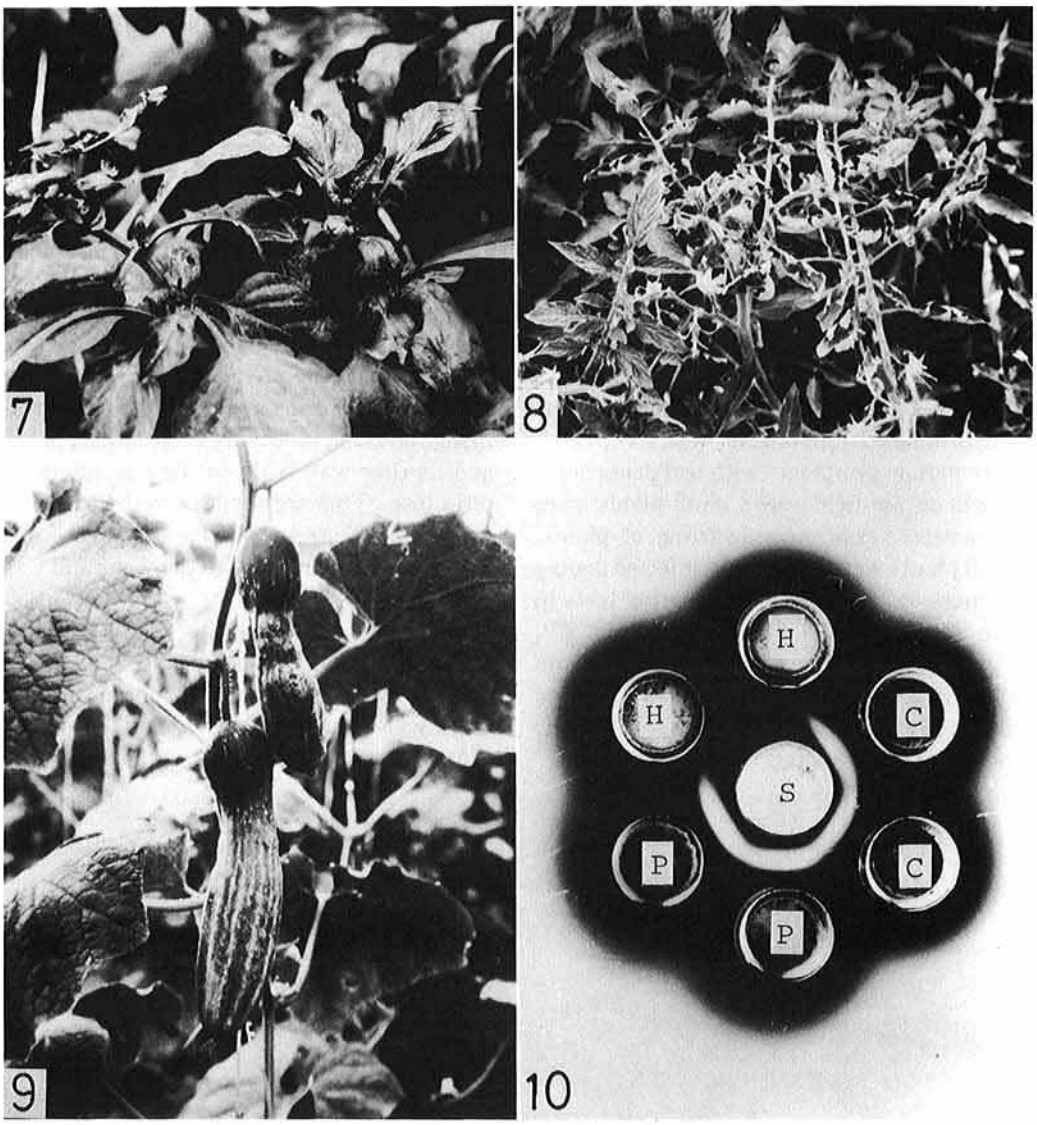


Plate 7. Severe mosaic and top necrosis symptom in sweet pepper caused by double infection of chilli veinal mottle virus and tomato spotted wilt virus
Plate 8. Leaf roll and mosaic symptoms in tomato plants caused by tomato spotted wilt virus
Plate 9. Deformed cucumber fruit caused by infection of zucchini yellow mosaic virus
Plate 10. Agar gel serological test with antiserum against zucchini yellow mosaic virus (S)
Other wells contained sap of healthy cucumber leaves(H), leaves infected with pumpkin isolate (P) and cucumber isolate (C)

Kampong Raja in Cameron Highlands. Since most sweet pepper was cultivated under plastic rain shelter, the incidence of the virus diseases was slightly less than that of chilli. In some area, however, the high rate of the virus disease infection was observed. Although several kinds of symptoms were observed like the case of chilli, mosaic symptom was very common (Plate 7). Nine samples of infected sweet pepper were collected, and viruses were detected from 4 samples. From the results of the identification tests of causal virus, 3 samples were found to contain CVMV alone while the other one was doubly infected with CVMV and TSWV.

3) Other crops

Incidence of virus diseases on other vegetable crops was also examined.

Some tomato plants showed mosaic symptoms in the area near chilli fields, and 2 samples collected from Bertam Valley were found to contain TMV-L alone. On the other hand, tomato plants in Pekan Nenas in Johor showed leaf roll or purple vein symptoms (Plate 8), and was found to be infected with TSWV.

A survey on virus diseases of eggplant was done in 2 fields, and CMV-O was detected from samples showing severe mosaic symptoms with necrotic ring spot in Subang Jaya in Selangor.

Spinach plants in the area near chilli fields in Bertam Valley showed severe mosaic symptoms and malformation of leaves, which was caused by infection with TSWV.

Many pumpkin plants showed mosaic symptoms in the field at Air Keroh and the incidence of virus diseases was almost 100%. Many cucumber plants in Subang Jaya showed mosaic symptoms and they generally produced many deformed fruit (Plate 9). The mechanically transmissible virus was isolated from both diseased plants, and it infected many species of Cucurbitaceae. The extracts of systemically infected leaves reacted positively with the antiserum against zucchini yellow mosaic virus (ZYMV) but not with antiserum against watermelon mosaic virus in SDS-immunodiffusion test (Plate 10). These results demonstrated that ZYMV was wide-spread in West Malaysia.

Summary

The occurrence of virus diseases on chilli, sweet pepper, tomato, eggplant, pumpkin and cucumber plants in West Malaysia were investigated. More than 80% of the cultivated chilli plants was found to be infected with mosaic disease. And the mosaic disease seemed to cause a severe loss of chilli yields. Out of 9 diseased chilli plants that infected test plants, 3 were found to contain chilli veinal mottle virus (CVMV) alone, while the others were doubly infected with the combination of CVMV and tomato spotted wilt virus (TSWV), CVMV and ordinary strain of cucumber mosaic virus (CMV-O), CVMV and tomato strain of tobacco mosaic virus, and of CVMV and chilli strain of CMV in proportion of 2, 2, 1 and 1. On sweet pepper, the incidence of the virus diseases was slightly less than that of chilli, though both CVMV and TSWV were detected. TSWV was also isolated from tomato and spinach, and produced severe mosaic and malformation of leaves. Zucchini yellow mosaic virus (ZYMV) was isolated from pumpkin and cucumber plants showing mosaic symptoms. Cucumber plants infected with ZYMV often produced many deformed fruit. Among the viruses isolated, CMV, CVMV and ZYMV have been reported to be transmitted by aphids in a non-persistent manner. On the other hands, TSWV has been reported to be transmitted by thrips in a persistent manner. Therefore, the protection from these insect vectors is most important for control of these virus diseases.

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chini yellow mosaic virus, respectively. We also thank Dr. M. Ishii, Vegetable and Ornamental Crops Research Station, Japan for his helpful suggestions.

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