

## Short Communication

# WATERMELON MOSAIC VIRUS OF PUMPKIN (*Cucurbita maxima*) FROM SULAWESI: IDENTIFICATION, TRANSMISSION, AND HOST RANGE

Wasmo Wakman<sup>a</sup>, M.S. Kontong<sup>a</sup>, D.S. Teakle<sup>b</sup>, and D.M. Persley<sup>c</sup>

<sup>a</sup>Indonesian Cereals Research Institute, Jalan Ratulangi 274, Maros 90154, Indonesia

<sup>b</sup>Department of Microbiology, The University of Queensland, Brisbane, Qld. 407, Australia

<sup>c</sup>Department of Primary Industries, Plant Protection Unit, Meiers Rd., Indooroopilly, Qld. 4088, Australia

## ABSTRACT

A mosaic disease of pumpkin (*Cucurbita maxima*) was spread widely in Sulawesi. Since the virus had not yet been identified, a study was conducted to identify the disease through mechanical inoculation, aphid vector transmission, host range, and electron microscopic test. Crude sap of infected pumpkin leaf samples was rubbed on the cotyledons of healthy pumpkin seedlings for mechanical inoculation. For insect transmission, five infective aphids were infected per seedling. Seedlings of eleven different species were inoculated mechanically for host range test. Clarified sap was examined under the electron microscope. Seeds of two pumpkin fruits from two different infected plants were planted and observed for disease transmission up to one-month old seedlings. The mosaic disease was transmitted mechanically from crude sap of different leaf samples to healthy pumpkin seedlings showing mosaic symptoms. The virus also infected eight cucurbits, i.e., cucumber (*Cucumis sativus*), green melon (*Cucumis melo*), orange/rock melon (*C. melo*), zucchini (*Cucurbita pepo*), pumpkin (*Cucurbita maxima*), water melon (*Citrulus vulgaris*), *Bennicosa hispida*, and *blewah* (*Cucurbita* sp.). Aphids transmitted the disease from one to other pumpkin seedlings. The virus was not transmitted by seed. The mosaic disease of pumpkin at Maros, South Sulawesi, was associated with flexious particles of approximately 750 nm length, possibly a potyvirus, such as water melon mosaic virus rather than papaya ringspot virus or zucchini yellow mosaic virus.

[Keywords: *Cucurbita maxima*; mosaic virus; disease transmission; host plants]

## INTRODUCTION

Mosaic disease of pumpkin (*Cucurbita maxima*) has been widely distributed in South Sulawesi in recent years, and has previously been reported from other places in Indonesia (Somowiyarjo, 1993; Semangun, 1994). Nineteen viruses are recorded to infect cucurbits (Brunt *et al.*, 1990), of which eight of them induced mosaic symptoms. The cucurbit viruses

have a world-wide distribution including China, Canada, USA, Europe, South Africa, Japan, Hawaii, and Cuba (Gibbs and Harrison, 1970; van Regenmortel, 1971; Lisa and Lecoq, 1984).

Some viruses that infected cucurbits were able to infect many other hosts, e.g., cucumber mosaic virus (CMV) also infected tobacco, chili, tomato, corn, banana, celery, bean, etc. (Walker, 1957; Semangun, 1994), cucumber, melon, squash, peppers, spinach, beets, crucifers, gladiolus, lilies, petunias, zinnias, and many weeds (Agrios, 1969). In Yogyakarta, at least four viruses infected cucurbits, i.e., CMV, watermelon mosaic virus-1 (WMV-1), watermelon mosaic virus-2 (WMV-2), and zucchini yellow mosaic virus (ZYMV) (Somowiyarjo, 1993). WMV-1, WMV-2, and ZYMV had flexious filamentous particle, and are transmitted by aphids and mechanically with plant sap (van Regenmortel, 1971; Lisa and Lecoq, 1984).

The cause of the mosaic disease of pumpkin in South Sulawesi has not been identified. The objectives of the study were to identify the virus and characterize the disease it caused.

## MATERIALS AND METHODS

### Mechanical Inoculation

Six leaf samples of infected pumpkin showing mosaic symptoms were collected from the fields at Maros and Barru districts of South Sulawesi in 1995/1996 and stored in a refrigerator. Seven trays filled with sterile soil were prepared. Twenty pumpkin seeds were planted in each tray and kept in screen cages. The germinated seedlings were prepared for the inoculation test. The cotyledons of the seedlings were dusted with carborundum just before inoculation.

Sap of pumpkin leaf sample was made by crushing 0.1 g leaf homogeneously in 2 ml distilled water in a mortar and pestle. The crude sap of each sample was immediately rubbed on the cotyledons of pumpkin seedlings using the pestle. The cotyledons were inoculated by distilled water instead of crude sap. The cotyledons were then washed from carborundum with distilled water and the inoculated pumpkin seedlings were kept in cages in a shaded place. The seedlings were examined for symptoms three times at weekly intervals, starting one week after inoculation.

#### Aphid Transmission

Aphids were collected from a field of healthy pumpkins and reared on mosaic affected pumpkins in a glasshouse. Twenty healthy pumpkin seedlings were prepared in a screen cage in a glasshouse and exposed to five aphids per seedling. As a control, 18 pumpkin seedlings were inoculated mechanically by using diseased plant extract as mentioned above and another 19 pumpkin seedlings were inoculated with distilled water as a control. Number of pumpkin seedlings showing mosaic leaf symptoms after inoculation was recorded weekly.

#### Host Range Test

Seeds of eleven different cucurbits were collected from markets and farmers and each was planted in two trays containing sterilized soils. The number of seeds planted per tray ranged from 20 to 30. Germinated seedlings were inoculated mechanically using the same method as mentioned above one week after planting. Only one set of trays was inoculated, and another set was used as a control. The mosaic symptom was recorded at 1 and 2 weeks after inoculation.

#### Electron Microscopic Test

The pumpkins with mosaic symptoms were tested for virus particles at the Indonesian Agricultural Biotechnology and Genetic Resources Research Institute, Bogor and the University of Queensland, Australia. Leaf samples of pumpkins (0.1 g) were triturated with 0.5 ml of 0.1 M phosphate buffer, pH 7, in a mortar using a pestle. The leaf extract was clarified in a microcentrifuge at 10,000 rpm for 5 minutes and the supernatant was collected. A 10- $\mu$ l drop of the supernatant was put on an electron microscope and grid for a minute, drained using filter paper, and examined for virus particles under an electron microscope.

#### Seed Transmission Test

Seeds were collected from a fruit of each of two different mosaic-affected pumpkin plants, and 220 and 147 seeds were planted respectively. The seedlings were observed for mosaic symptoms up to one month after planting.

### RESULTS AND DISCUSSION

Leaves of infected pumpkin plants showed green and yellow green systemic mosaic, vein banding and sometime malformation (Fig. 1). Infected plants produced abnormal flowers with undeveloped or incompletely opened petals. The abnormal flowers were mostly folded and produced no fruits. Both transmission methods, mechanical and aphid vector inoculations could be used to transmit the mosaic disease to healthy pumpkin seedlings (Fig. 2).

Observation on six diseased leaf samples collected from six different infected pumpkin plants from fields showed that mosaic was transmitted mechanically to



Fig. 1. Mosaic of pumpkin leaf in South Sulawesi, Indonesia.



Fig. 2. Pumpkin seedlings showing mosaic symptoms following mechanical inoculation.

**Table 1. Mechanical transmission of six pumpkin leaf samples from six different infected pumpkin plants to healthy pumpkin seedlings.**

Pumpkin mosaic sample	No. of inoculated pumpkin seedlings	No. of infected pumpkin seedling	Infection (%)
Leaf sample 1	20	20	100
Leaf sample 2	18	16	89
Leaf sample 3	19	12	63
Leaf sample 4	20	19	95
Leaf sample 5	17	13	76
Leaf sample 6	9	8	89
Distilled water	9	0	0

**Table 2. Mechanical and aphid vector transmission of the pumpkin mosaic virus.**

Inoculation method	No. of pumpkin seedlings inoculated	No. of infected pumpkin seedlings	Infection (%)
Mechanical	18	18	100
Aphids	20	6	30
Control (uninoculated)	19	0	0

pumpkin seedlings with infection rates ranging from 63 to 100% (Table 1). High transmission rates up to 100% were found for mechanical inoculation, while aphid transmission resulted in a low transmission rate (Table 2). The pumpkin mosaic infected 8 of 11 cucurbits inoculated mechanically, i.e., *Cucumis sativus*, *Cucumis melo* (green flesh), *C. melo* (orange flesh), *Cucurbita pepo*, *Cucurbita maxima*, *Cucurbita* sp. (blewah), *Citrullus vulgaris*, and *Bennicosa hispida* (Table 3).

Electron microscopic test indicated that the pumpkin mosaic disease agent possessed rod shaped virus-like

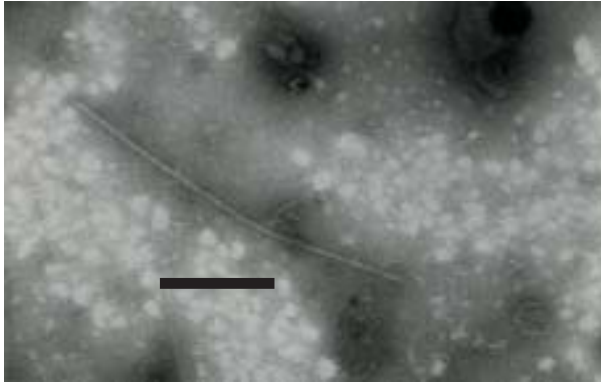
particles of a length approximately 750 nm (Fig. 3). When 367 seeds of two fruits of infected pumpkins were planted, none of the seedlings showed mosaic symptoms.

Mosaic symptoms of pumpkins in South Sulawesi were transmitted by aphids (*Myzus persicae*) to healthy pumpkin seedlings. The disease was also transmitted mechanically to 8 of 11 cucurbits inoculated. The finding indicated that the disease was caused by a virus. Brunt *et al.* (1990) reported that of 19 viruses infected cucurbits, 5 were transmitted both mechanically and by aphid vector,

**Table 3. Percentage of infected cucurbits following inoculated by pumpkin mosaic disease.**

Leaf extract	No. of seedling inoculated		No. of control seedlings (uninoculated)	
	Total	Infected (%)	Total	Infected (%)
<i>Cucumis sativus</i>	20	80	20	0
<i>Cucumis melo</i> (green flesh)	30	10	30	0
<i>C. melo</i> (orange flesh)	30	7	30	0
<i>Cucurbita pepo</i>	11	91	<sup>1)</sup>	<sup>1)</sup>
<i>Cucurbita maxima</i>	20	95	20	0
<i>Citrullus vulgaris</i>	20	95	20	0
<i>Bennicosa hispida</i>	20	75	20	0
<i>Cucurbita</i> sp.	23	30	14	0
<i>Cucumis</i> sp.	27	0	32	0
<i>Luffa acutangula</i>	20	0	20	0
<i>Momordica charantia</i>	15	0	10	0

<sup>1)</sup>No data seedling



**Fig. 3.** Electron micrographs of particles associated with pumpkin mosaic viruses (Bar = 200nm).

i.e., bean yellow mosaic potyvirus (BYMV), cucumber mosaic cucumovirus (CMV), papaya ringspot virus (PRSV-W), watermelon mosaic virus-2 (WMV-2), and zucchini yellow mosaic potyvirus (ZYMV). Persley (1994) reported that three viruses caused mosaic disease on cucurbits in Queensland, i.e., PRSV-W, WMV-2, and ZYMV.

Papaya is widely grown in South Sulawesi. However, PRSV was not found and has never been reported, suggesting that no PRSV exists in South Sulawesi. Zucchini was never introduced nor grown in South Sulawesi, indicating that ZYMV may not have been introduced into South Sulawesi.

Electron microscopic observation found that the virus particles of pumpkin mosaic from South Sulawesi was a flexuous filament rod shape. Of the five viruses infecting cucurbits, three are flexuous filament rod shape, e.g., PRSV-W, WMV-2, and ZYMV (Brunt *et al.*, 1990). The pumpkin mosaic serologically related to WMV-2.

A survey of 34 samples of *C. maxima* in Yogyakarta found 20 samples similar to WMV-2, 9 samples related to CMV, 2 samples closed to PRSV-2, 2 samples belonging to ZYMV group, and 4 samples with double infection of WMV-2 and CMV (Somowiyarjo, 1993). *C. maxima* was infected by WMV-2 ten times more frequently than by ZYMV or PRSV-W in the field.

## CONCLUSION

Based on the morphology of the virus particle, transmission, and host range, it was concluded that the mosaic disease of pumpkin at Maros was caused by virus particles belonging to the potyvirus group. It was thought to be WMV-2 rather than PRSV-W or ZYMV. This is the first time that the virus mosaic disease of cucurbits has been reported in South Sulawesi.

## ACKNOWLEDGEMENT

We would like to thank Mr. Muchsin of Indonesian Agricultural Biotechnology and Genetic Resource Research Institute and Mr. Rick Webb of the University of Queensland, Australia for help with electron microscopic test and taking micrographs of the virus-like particles.

## REFERENCE

- Agrios, G.N. 1969. Plant pathology. Academic Press, New York. p. 629.
- Brunt, A., K. Crabtree, and A. Gibbs. 1990. Viruses of tropical plant. CAB International, UK. p. 707.
- Gibbs, A.J. and B.D. Harrison. 1970. Cucumber mosaic virus. CMI/AAB. Description of plant viruses No. 1.
- Lisa, V. and H. Lecoq. 1984. Zucchini mosaic virus. CMI/AAB Description of plant viruses No. 282.
- Persley, D. 1994. Disease of vegetable crops. Department of Primary Industries, Queensland. 100 p.
- Semangun, H. 1994. Penyakit-penyakit tanaman hortikultura di Indonesia. Gadjah Mada University Press, Yogyakarta. 850 hlm.
- Somowiyarjo, S. 1993. Detection and identification of cucurbit viruses in Yogyakarta. Ilmu Pertanian (Agric. Sci.) 3: 657-663.
- van Regenmortel, M.H.V. 1971. Watermelon mosaic virus. CMI/AAB. Description of plants viruses. No. 63. 4 p.
- Walker, J.C. 1957. Plant pathology. Mc Graw-Hill Book Co. Inc., New York. p. 707.