DENDROBIUM MOSAIC VIRUS

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INTRODUCTION

A new virus was isolated from *Dendrobium*, which showed markedly a distinct mosaic in the leaves, in an orchid nursery of Okayama, in May 1967. Since then this disease was also found in some other districts in the western part of Japan. The symptoms were very different from those of the three known viruses, Cucumber mosaic virus(4,11), Cymbidium mosaic virus(3,5,8-10) and Bacilliform virus(1,12) of *Dendrobium*. Virus particles detected from the diseased leaves are flexuous rods, about 750 nm in length and the particles of this size have first been found among viruses in *Orchidaceae* plants. The virus was newly named Dendrobium mosaic virus (DeMV) owing to the characteristics of the symptoms on *Dendrobium*. This paper mainly deals with host range, symptoms, transmission, physical properties and electron microscopy of the virus.

MATERIALS AND METHODS

An isolate of DeMV used in this studies was isolated by sap inoculation from the diseased plants of *Dendrobium* sp. (nobile) collected in Okayama, Japan. It shows a distinct mosaic and concentric green ring patterns on the leaves which are very different from those of the other known *Dendrobium* viruses(1,3-5,8-12). The original diseased plants were maintained in a greenhouse for inoculum source. Sap inoculation was conducted by carborundum rubbing methods.

Virus particles were observed under a Hitachi HS-6 and a Hitachi HU-12 electron microscopes. Preparations for observation were made by means of dip methods or by direct negative stain method using 2% phosphotungstic acid. Small pieces of the young leaves of the diseased *Dendrobium* showing mosaic symptoms were fixed with 5% glutaraldehyde for 1 hr and then by chilled 1% osmium tetroxide for 1 hr.

After fixation, the tissues were dehydrated in a graded series of ethanol and absolute acetone, and embedded in a mixture of Epon 812, MNA and DDSA in a ratio of 3:2:1 (volume).

Thin sections were cut by a Poter-Blum MT-1 microtome and stained with uranyl acetate and lead citrate. For the partial purification of this virus, the leaves of artificially diseased *Dendrobium* were ground

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in a grindbowl with 5 v/w of 0.1 M phosphate buffer, pH 7.6 and the juice was expressed through cheesecloth. The crude juice was treated with 1/3 volume of chloroform for 3 min., and centrifuged for 10 min. at 3,000 rpm. The supernatant fluid was further centrifugated at 7,000 g for 10 min. and then centrifugated 105,000 g for 60 min. After two cycles of low- and high-centrifugation, the pellets were suspended in 0.002 M phosphate buffer, pH 7.6. The partially purified virus was used as preparation for electron microscope.

RESULTS

1) Symptoms in naturally infected Dendrobium plants.

Symptoms in the leaves are characterized by the distinct mosaic and concentric ring patterns with slender green line (Plate I, 1-3). In the young leaves, the symptoms of light mottling appear. The margins of the green area appeared comparatively clearly. No flower showed symptoms.

2) Host range and symptoms

DeMV caused systemic infection only in Dendrobium and sometimes local lesions in Chenopodium amaranticolor and C. quinoa. Symptoms of the host plants are as follows:

(a) Dendrobium Chlorotic spots appeared on the younger inoculated leaves 2-3 weeks after inoculation. The chlorotic spots extended and elongated and sometimes became spindle-shape, and then extended into coalescence in all the leaves. In affected leaves spindle-shape green ring patterns or the ring enclosing normal green spots remained and showed a well-defined mosaic (Plate I, 5).

The virus became systemic and chlorotic spots appeared first on the new growth and then extended and coalesced in all the leaves. In the affected areas, clear green spot and diamond-shaped green patterns remained and the symptoms showed the distinct mosaic (Plate I, 4, 6). The elongated, distinctly concentric, green ring patterns also developed. The chlorotic spots occasionally developed first between the veins on the upper part of the leaves and then extended downward to the lower part of the leaf along the veins (Plate I, 7). Symptoms seldom appeared first in the lower part on the leaves.

In the leaves on the newly developing shoots after inoculation, symptoms appeared first in the second, third or fourth leaves, and became the distinct mosaic or concentric green patterns as previously described. No flower showed symptoms.

(b) C. amaranticolor and C. quinoa. These plants were not in-
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infected by the diseased plant sap, but when these plants were inoculated with partially purified and concentrated virus preparations, a few local lesions were formed on the inoculated leaves. On *C. amaranticolor*, local light green spot appeared on the inoculated leaves when they turned yellow, without systemic infection (Plate 1, 8). On *C. quinoa*, a few chlorotic spots developed on the inoculated leaves.


3) **Transmission**

DeMV is easily transmitted by plant sap. Aphid transmission experiments were done using *Dendrobium* seedling plants as the source and indicator plants. When the aphids were placed on *Dendrobium* overnight, many aphids escaped or some died. So the most transmission of the virus by the aphids failed. But when the aphids were allowed to probe, the virus was transmitted by *Myzus persicae* at a low rate.

4) **Physical properties**

The physical properties *in vitro* of DeMV in the expressed sap of diseased *Dendrobium* were examined using *Dendrobium* seedlings as the indicator plants. Table 1 shows the results. The virus remained infective at 50°C for 10 min. exposure, but was inactivated at 55°C. It was inactivated in the ages of 4 to 8 days at 20°C.

5) **Virus particles**

The particle's morphology of DeMV was long and flexuous rods. The length is shown in Fig. 1. The modal length of virus particles
measured in sap from the diseased leaves was about 750 nm, and all particles were about 13 nm in width.

**TABLE 1**

**Stability of Dendrobium mosaic virus in crude sap**

<table>
<thead>
<tr>
<th>Thermal inactivation point (10 min.)</th>
<th>Control</th>
<th>40°C</th>
<th>50°C</th>
<th>55°C</th>
<th>60°C</th>
<th>65°C</th>
<th>70°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5/5</td>
<td>5/5</td>
<td>3/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Longevity <em>in vitro</em> (20°C)</th>
<th>Control</th>
<th>1 day</th>
<th>2 days</th>
<th>4 days</th>
<th>8 days</th>
<th>16 days</th>
<th>1 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3/3</td>
<td>3/3</td>
<td>1/3</td>
<td>1/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
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</tbody>
</table>

Denominator indicated the number of test plants, the numerator indicated the number that became infected.

![Graph of particle length distribution](image)

**Fig. 1.** Particle length of Dendrobium mosaic virus

6) **Ultrastructure of infected Dendrobium cells**

Plates III and IV show the ultrastructures of cell of *Dendrobium* infected with DeMV. In cytoplasm of infected plant cells, pinwheel inclusions were observed. They were sometimes orientated perpendicularly to the cell wall (Plate IV, 1, 2). Virus particles were dispersed in the cytoplasm but the arranged moss was not found.

**DISCUSSION**

The three viruses, Cymbidium mosaic virus(3, 5, 8–10), Cucumber mosaic virus(4, 11) and Bacilliform virus(1, 12), are commonly known as virus diseases in *Dendrobium* orchids. Symptoms on the leaves of *Dendrobium* infected with DeMV are characterized by the distinct mosaic
and concentric green ring patterns with comparatively clearly developed margins. These symptoms seemed to be very different from those induced by the other three viruses in Dendrobium above mentioned. So the characteristics of these symptoms are useful for diagnosis of DeMV in Dendrobium. DeMV is easily transmitted to Dendrobium by diseased plant juice, but the plants of Orchidaceae such as Cattleya, Cymbidium, Miltonia, Oncidium, and Zygopetalum were found to be insusceptible, and the virus was also not infectious to the many other plants tested outside Orchidaceae. Now only Dendrobium was systemically susceptible plant to DeMV.

In the host range of DeMV reported in the author’s earlier paper(6), C. amaranticolor and C. quinoa were found to be insusceptible, but when the plants were inoculated with partially purified and concentrated virus preparations, a few local lesions were formed. Particles of the DeMV are flexuous rods about 750 nm in length and about 13 nm in width, and this virus was a new one in which these morphological particles were first found in the plants of Orchidaceae. Since then Bean yellow mosaic virus of similar particles in size was isolated from Calanthe by the author (1972)(7).

DeMV was transferred by aphid, Myzus persicae, in the non-persistent manner, but the aphids seem not to like to feed on the leaves of Dendrobium plants.

In cytoplasm of the diseased plant cells, the virus induced pinwheel inclusions which were morphologically indistinguishable from those reported for many other viruses in the Potyvirus group(2). From the results previously described, DeMV seemed to belong to the Potyvirus group.

**SUMMARY**

A new virus disease, Dendrobium mosaic, was first found in an orchid nursery of Okayama, in May 1967. Since then this disease was also found in some other districts in the western part of Japan.

Well-defined mosaic and concentric green ring patterns are the marked characteristic symptoms in Dendrobium. No symptoms were noticed in flowers. These symptoms are very different from the mottle mosaic caused by Cymbidium mosaic virus, with flower color breaking, mild chlorotic ring mottle caused by Cucumber mosaic virus and leaf spot or chlorotic fleck caused by Bacilliform virus.

DeMV is easily transmitted by diseased plant juice, and the virus also transmitted by aphid (Myzus persicae) in the non-persistent manner. It causes the systemic mosaic on Dendrobium, and sometimes formed some local lesions on the inoculated leaves of C. amaranticolor and C. quinoa, but not Cymbidium, Cattleya and its hybrids, Miltonia, Oncidium.
and *Zygopetalum*. All other plants tested, 44 species in 12 families, such as *Nicotiana tabacum* (White Burley, Samsun), *N. glutinosa*, *Datura stramonium*, *Lycopersicon esculentum*, *Gomphrena globosa*, *Cucumber sativum*, *Tetragonia expansa* are found to be insusceptible to the virus. DeMV in diseased plant juice is inactivated at the temperatures of 55-60°C for 10 minutes exposure and in 4 to 8 days aging at 20°C.

The normal length of virus particles observed in negatively stained extracts of infected leaves was about 750 nm and about 13 nm in diameter. In ultra-thin sections of diseased *Dendrobium* leaf tissues, pin-wheel inclusions are observed in the cytoplasm of infected cells, and virus particles were also observed to disperse in the cytoplasm.

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**REFERENCES**

Plate I. Symptoms caused by Dendrobium mosaic virus in *Dendrobium* and *Chenopodium amaranticolor*.

1-3. Mosaic and/or green ring patterns on leaves from naturally infected *Dendrobium*.

4, 6, 7. Mosaic on leaves of *Dendrobium* systemically infected with DeMV.

5. Green ringspotting on the inoculated leaf of *Dendrobium* infected with DeMV.

8. Local lesions on the inoculated leaf of *C. amaranticolor*. 
Plate II. Electron micrographs of particles of Dendrobium mosaic virus.
1. Particles of DeMV in dip preparation. Scale bar represents 300 nm.
2. Negatively stained particles of DeMV in dip-preparation mounted in PTA. Scale bar represents 100 nm.
Plate III

Plate III. Ultrastructure of mesophyll cells of *Dendrobium* infected with DeMV.
1. Pinwheel inclusions in DeMV-infected cell.
2. Longitudinally sectioned pinwheel inclusions and particles in DeMV-infected cell.
Plate IV. Ultrastructure of mesophyll cells of *Dendrobium* infected with DeMV.
1, 2. Longitudinally sectioned pinwheel inclusion attached to wall.
3. Pinwheel inclusions induced by DeMV in *Dendrobium* mesophyll cell.