A strain of cucumber mosaic cucumovirus causing mosaic in marigold in India

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ABSTRACT: A strain of cucumber mosaic cucumovirus causing marigold mosaic was partially characterized. The virus was isolated from diseased marigold (Tagetes erecta L.) plant growing at and around Aligarh. Diseased plants showed mosaic, mottling and stunting. Infected leaf tissue under electron microscope showed the presence of isometric virus particles measuring about 29 nm in diameter, resembling those of cucumoviruses. There was enhanced trapping and decoration of virions with antisera to strains of cucumber mosaic cucumovirus, namely, soybean stunt (CMV-SS) and pea strain (CMV-P) in immunoelectron microscopic studies. There was neither enhancement of trapping nor any perceptible decoration of virions with antiserum to CMV-KS cucumovirus. Host range, biophysical properties and non-persistent transmission through Aphis gossypii, A. craccivora and Myzus persicae also supported the diagnosis of the virus as a strain of cucumber mosaic cucumovirus.

Key words: Marigold mosaic, cucumovirus, purification, IEM

Marigold (Tagetes erecta L.) is an important ornamental plant grown in homes, gardens and fields in whole of north and some other parts of India. It is economically important for its showy flowers, tagetes oil and anti-nematicidal properties. It is a host to a few plant viruses some of which cause mosaic disease (Hanson et al., 1951; Joshi and Dubey, 1972; Usman et al., 1972; Sang and Varma, 1975; Naqvi et al., 1981; Rahman and Rao, 1992), tomato leaf curl virus (Sastry, 1984) and potato yellow dwarf virus (Lockhart, 1989).

Symptoms of marigold mosaic disease occurring at Aligarh comprised mosaic and mottling in the beginning followed by distortion of leaves leading to bare midribs in the later stages. Due to the great economic significance of marigold in India and incomplete characterization of the mosaic causing virus(es), attempts were made to identify and characterize the virus causing mosaic at Aligarh.

MATERIALS AND METHODS

Leaves of naturally infected plants of Tagetes erecta showing mosaic mottling and stunting were used for aphid and sap inoculation studies. Pure virus culture was maintained on Nicotiana glutinosa while virus was propagated on Nicotiana tabacum var. CTRI special type FCV, N. tabacum var. Harison Special and N. rustica. Different plant species used to study the host range of the virus were grown in an insect proof glasshouse in sterilized soil in pots. Chenopodium amaranticolor, a good indicator host, produced chlorotic local lesions and was used to check susceptibility of other inoculated plant species through back inoculation.

Vector transmission was studied by employing aphid species, namely, Aphis gossypii Glov., A. craccivora Koch. and Myzus persicae Sulz. and whitefly, Bemisia tabaci Genn. Aphids and whiteflies used for transmission were starved for 2h, then given acquisition feeding of 2 min to 12 h on diseased plants of N. glutinosa followed by inoculation feeding on healthy plants for 2 min to 24 h.

The seeds from infected Tagetes erecta L. and Nicotiana rustica were collected and sown in autoclaved soil to study seed transmission. After seedling emergence, the plants were observed till 5-6 weeks and sprayed with 0.02% cypermethrine, an insecticide to prevent insect infestation. Leaf tissues of these plants were also assayed on C. amaranticolor.

Electron microscopic studies were carried out by using clarified virus concentrate prepared according to Christie et al. (1987). Since virus particles were observed only in the preparation made by using 0.05M phosphate buffer, only this concentration was used for immunoelectron microscopic (IEM) studies. Antisera

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to different plant viruses, namely, potato leaf roll virus (PLRV), cowpea mosaic virus (CPMV), cucumber mosaic virus (soybean stunt strain) (CMV-SS), pea strain (CMV-P) and sweet pepper strain (CMV-KB) were used in the IEM studies. All the antisera except PLRV and CPMV were obtained from Dr. Z. Maat (Netherlands). All the antisera were diluted 200 fold with 0.88% NaCl solution before use. Trapping, decration and counting of trapped virions was done according to Garg and Khurana (1992, 1993).

The present virus isolate was purified following Walkey (1985) with some modifications. The 200 g infected leaves of *N. glutinosa* were homogenized in 400 ml of 0.05M phosphate buffer (pH 7.0) containing 0.1% thioglycolic acid and 0.1% sodium sulphite. Clarification of the extract and virus precipitation with 6% PEG was done according to Walkey (1985). The virus isolate was further purified in 10-40% sucrose gradient columns at 24,000 rpm for 2 h in an ultracentrifuge. The light scattering zone was collected and centrifuged at 35,000 rpm for 2h. The final pellet was suspended in 2 ml of phosphate buffer (0.05M, pH 7.0).

Studies on biophysical properties, namely, thermal inactivation point (TIP), longevity *in vitro* (LIV) and dilution end point (DEP) were carried out by using sap from infected leaves of *N. glutinosa* by employing the standard procedures (Noordam, 1973).

RESULTS

Host Range

The virus had a moderate host range and infected members of Chenopodiaceae, *Vigna sinensis*, *Vigna radiata* and *V. mungo* locally after 4-5 days of mechanical inoculation. It caused systemic infection in many species of Solanaceae and only four species of Cucurbitaceae 10-14 days after inoculation.

The members of Chenopodiaceae, viz., *Chenopodium amaranticolar* Coste and Reyn., *C. albam* L., *C. murale* L. and *C. quinoa* produced chlorotic local lesions while *Beta vulgaris saccharifera* L. produced necrotic local lesions. *Tagetes erecta* L. was observed with systemic mild mosaic and mottling symptoms. Three species of *Cucurbitaceae* i.e. *Citrullus vulgaris* var. *fistulosus*, *Cucumis melo utilissmus* and *C. sativus* produced systemic mosaic symptoms and plants become wilted 5-6 days after symptom expression, while later two species also produced discrete local lesions on the cotyledons which later on became necrotic. One species of this family, *Momordica charantia* L. was associated with systemic mild mosaic and leaf deformation. *Vigna mungo* L. Hepper and *V. radiata* L. Wilezek produced black local lesions while *V. sinensis* (L.) Savi ex Hasak was observed with red local lesions. The species of Solanaceae, viz., *Datura metal* L., *D. stramonium*, *Lycopersicum esculentum*, *Nicotiana alata*, *N. clevelandii*, *N. debnnyi*, *N. glutinosa*, *N. longiflora*, *N. occidentalis*, *N. rustica*, *N. tabacum* var. Harison Special type FCV, *N. tabacum* var. Jayasri type FCV., *N. tabacum* var, Samsun type Turkish and *Physalis peruviana* L. produced systemic mosaic symptoms. *N. glutinosa* and *P. peruviana* also showed shoe-string symptoms while wilting with necrosis was observed in *N. megalosiphon* plants. *N. plumbaginifolia* was associated with mild mosaic and leaf deformation symptoms. Leaf deformation was also observed with mosaic in *N. rustica*.

Fig. 1. Morphology of virions (A), and moderate decoration of virions with antisera to CMV-SS/CMV-P. Despite prior fixation of the trapped virions with glutaraldehyde, partial degradation of virions during decoration is still discernible (B). Bar-250 nm

**Transmission**

The virus was transmitted by *A. gossypii*, *A. craccivora* and *M. persicae*, while *B. tabaci* was unable to transmit it. The virus was acquired within 2-3 min of acquisition and transmitted by 2-3 min inoculation feeding. No latent period was required.

The present virus isolate was found to be 10% seed transmissible when the young infected seedlings of *T. erecta*, *N. glutinosa* and *N. rustica* were tested on *C. amaranticolor*.

**Biophysical properties**

The virus retained infectivity in crude sap for 72 h at room temperature (25±5°C) and 162 h at 4°C. The dilution end point of the virus was 10⁻⁶, and the thermal inactivation point was 60°C.

**Table 1.** Trapping index° of the virus with different antisera.

<table>
<thead>
<tr>
<th>Coating serum/antiserum</th>
<th>No. of virions trapped</th>
<th>Trapping index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal serum (control)</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>No coating (control)</td>
<td>7</td>
<td>3.5</td>
</tr>
<tr>
<td>CMV-K8</td>
<td>8</td>
<td>4.0</td>
</tr>
<tr>
<td>CMV-SS</td>
<td>16</td>
<td>8.0</td>
</tr>
<tr>
<td>CMV-P</td>
<td>16</td>
<td>8.0</td>
</tr>
<tr>
<td>PLRV</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>CPMV</td>
<td>3</td>
<td>1.5</td>
</tr>
</tbody>
</table>

\[ a = \frac{\text{No. of virions on antisera coated grid}}{\text{No. of virions on normal serum coated grid}} \]

**Electron Microscopy**

Transmission electron microscopy showed the presence of isometric virus particles c.29 nm in diameter (Fig. 1A). Virions showed a small hollow central area made visible by stain entry.

**Immunoelectron Microscopy**

Table 1 shows that there was enhanced trapping with antisera to cucumber mosaic virus strains CMV-SS and CMV-P. There was also moderate decoration with these antisera (Fig. 1B), but no perceptible decoration with antiserum to CMV-K8.

**DISCUSSION**

First report of a virus causing mosaic disease on marigold was of Hanson *et al.* (1951) who studied only transmission behaviour of the virus and its host range. Later on, virus associated mosaic of marigold was reported in India by Joshi and Dubey (1972), Usman *et al.* (1972), Sang and Varma (1975), Naqvi *et al.* (1981), and Rahman and Rao (1992). The virus reported by Usman *et al.* (1972) was transmissible only through graft and not through aphids. While the disease studied by Naqvi *et al.* (1981), and Rahman and Rao (1992) were caused by a flexuous virus, the one studied by Sang and Varma (1975) was caused by an isometric virus which was proposed to be a cucumovirus. Earlier, Joshi and Dubey (1972), on the basis of host range and biophysical studies, had also proposed the virus to be a cucumovirus. Results presented in the present paper also reveal that the virus is a cucumovirus. However, our serological studies based on immuno-electron microscopy further reveal that the virus has seroaffinity with CMV-SS and CMV-P and not with CMV-K8.

Various strains of CMV have been placed in two distinct groups termed ToRS and DTL (Devergne and Cardin, 1973, 1975). CMV-K8 has been reported to be more closely related to CMV-To which falls in the CMV group ToRS (Tobias *et al.*, 1972). CMV-P and CMV-SS are closely related to the type strain of CMV (Tochihara and Tamura, 1976; Takahashi *et al.*, 1980) which belongs to the CMV group DTL. Thus, we conclude that the virus causing marigold mosaic appears to belong to the DTL group of CMV. However, in contrast to the strain reported by Sang and Varma (1975), our strain failed to infect *Zinnia elegans*, *Cucurbita pepo*, *Calendula officinalis* and *Antirrhinum majus*. 
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REFERENCES


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