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Color Breaking Syndrome of *Matthiola incana* Caused by Double Infection of Cucumber Mosaic Virus and Turnip Mosaic Virus

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오이 모자이크 바이러스와 순무 모자이크 바이러스의 복합감염에 의한 스톡의 꽃잎얼룩무늬병

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ABSTRACT: In 1995, we collected stock (*Matthiola incana*) plants causing mosaic symptoms on leaves and color breakings on flowers in Daekwallyong, Korea. Two viruses were isolated from the infected plants, and identified as cucumber mosaic virus (CMV) and turnip mosaic virus (TuMV) by experiments of host range, serology and electron microscopy. Each of the virus did not produce the same symptoms on the stock seedlings as naturally infected plants caused. When the viruses were coinoculated to the stock seedlings, however, severe mosaic symptoms were observed on leaves, and then the color breakings were expressed on flowers.

***Key words*: *Matthiola incana*, color breaking, CMV, TuMV, double infection.**

Stock (*Matthiola incana*) is widely grown ornamental plants for cutting and bedding flowers. So far, cauliflower mosaic virus (CaMV) and turnip mosaic virus (TuMV) have been reported as causal viruses of stunting, mosaic and severe leaf malformation on *M. incana* (1). In 1995, we collected stock plants causing severe mosaic on leaves and color breaking on flowers in Daekwallyong, Korea (Fig. 1). In this paper, we report the characterization and identification of the two viruses, cucumber mosaic cucumovirus (CMV) and turnip mosaic potyvirus (TuMV) isolated from the infected stock plants.

Isolation of the viruses. Crude sap of the diseased stock leaves was transferred mechanically to several indicator plants including *Chenopodium quinoa*, in which the viruses induced two types of necrotic- and chlorotic-local lesions. Two viruses were isolated by three successive single isolation from each necrotic- and chlorotic-lesion on *C. quinoa*. Spherical and filamentous virus particles, tentatively named as Mi-S and Mi-F were observed from the necrotic- and chlorotic-lesions of *C. quinoa* by electron microscopic examination, respectively. And then, the single lesions from the each plant were propagated in *Nicotiana glutinosa* and *Raphanus sativus*, respectively.

Host range. Two viruses propagated in *N. glutinosa* and *R. sativus* were inoculated mechanically to 26 plant species of 9 families, and the inoculated plants were kept in a greenhouse at 20~25°C. Among them, twenty-three species of 9 families were infected by Mi-S. The plants showed local symptoms on inoculated leaves but not infected systemically were as follows; *C. amaranticolor, C. quinoa, Datura stramonium, Vigna sesquipedalis, V. radiata* and *Vicia faba*. Mi-S systemically infected *N. glutinosa, N. rustica, N. benthamiana, N. clevelandii, N. occidentalis, N. tabacum* 'Ky-57', 'Xanthinc', 'Samsun' and 'Bright Yellow', *Lycopersicon esculentum, Physalis floridana, Capsicum annuum, Cucumis sativus, Tetragonium expansa, Zea mays, Sesamum indicum* and *Perilla frutescens*. On the other hand, nine species of 5 families were infected by Mi-F. Only *T. expansa* induced local symptoms on inoculated leaves. The virus caused systemic infection on *C. amaranticolor, C. quinoa, N. clevelandii, N. occidentails, N. benthamiana, P. floridana, R. sativus* and *S. indicum*.

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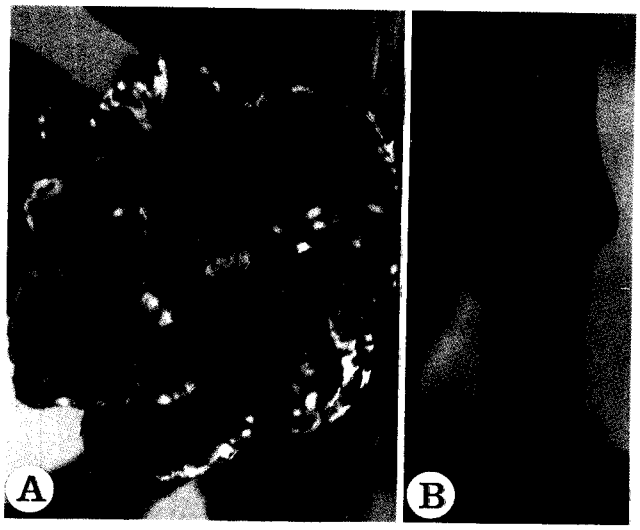

Fig. 1. Stock (*Matthiola incana*) plant naturally infected with cucumber mosaic virus (CMV) and turnip mosaic virus (TuMV) showing color breaking on flower (A) and mosaic symptom on leaf (B).

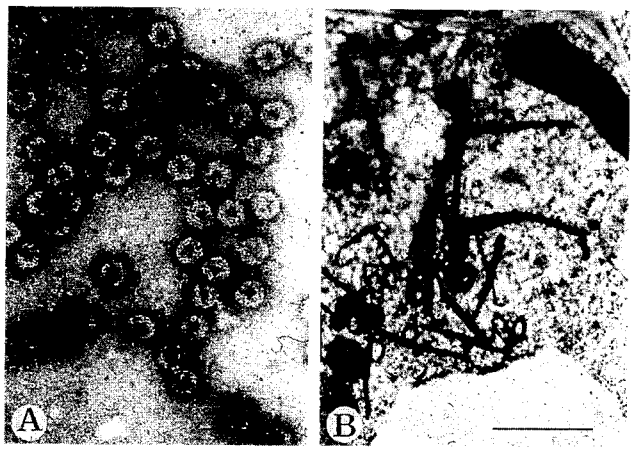

Fig. 2. A: Electron micrograph of virus particles purified from *N. tabacum* 'Ky-57' infected with cucumber mosaic virus (CMV) stock isolate. Virus particles stained with uranyl acetate. Bar represents 100 nm. B: Thin section of *Brassica rapa* infected with turnip mosaic virus (TuMV) stock isolate. Numerous inclusions of cylindrical, scroll and laminated shapes scattered throughout the cytoplasm. Bar represents 500 nm.

Among them, *C. amaranticolor, C. quinoa, N. benthamiana, N. occidentails, N. clevelandii* and *P. floridana* also produced local symptoms on inoculated leaves.

Serology. Agar gel double diffusion test (5) was used to detect the Mi-S and Mi-F. The purified preparation (7) of Mi-S strongly reacted with antiserum to CMV-D (ATCC PVAS-163), but no reactions were detected with antisera to brome mosaic virus (BMV, ATCC PVAS-178), carnation ringspot virus (CRSV, ATCC PVAS-21), turnip yellow mosaic virus (TYMV, ATCC PVAS-255), cowpea mosaic virus (CpMV, ATCC PVAS-248) and bean pod mottle virus (BPMV, ATCC PVAS-564) in 1% agarose gels. Serological relationship of the purified Mi-F preparation (3) was also tested to antisera of four potyviruses by double diffusion method using 0.7% agarose gel containing 3% sodium dodecyl sulfate (SDS). Mi-F clearly reacted with antisera to TuMV-cqs (2) and potato virus Y (PVY) (6), but not with antisera to tobacco etch virus (TEV, ATCC PVAS-69) and sugarcane mosaic virus (SCMV, ATCC PVAS-115).

Electron microscopy. The purified preparations of Mi-S fixed previously with 2% glutaraldehyde were made by negative staining with 1% uranyl acetate. Observation was done by a Zeiss electron microscope EM 109. Electron microscopy revealed that purified virus preparations contained small spherical particles with a diameter of about 30 nm (Fig. 2A). For ultrastructural studies of Mi-F, small pieces of leaf tissue from the Mi-F- infected *Brassica rapa* were fixed with 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 2 h and were followed by post-fixation with 1% osmic acid in Dalton's solution (pH 7.2) at 4 °C for 2 h. After dehydration in an ethanol series, these tissues were embedded in epon resin. Ultrathin sections were made with a diamond knife on a Sorvall ultramicrotome MT 6000. Sections were stained with uranyl acetate and lead citrate, and then examined under a Hitachi electron microscope H-800. The cytoplasm of infected epidermal, palisade, and mesophyll cells of *B. rapa* was highly vesiculated and contained pinwheel, scroll and laminated inclusion bodies (Fig. 2B), suggesting to be type III in Edwardson's potyvirus classification (4).

Inoculation to *M. incana* seedlings. The stock seedlings inoculated with Mi-S or Mi-F at the five-leaf stage, propagated under greenhouse conditions, developed chlorotic local lesions on the inoculated leaf within 6 days. Systemic symptoms first appeared at 9 to 12 days after inoculation as vein clearing, and followed by mild mosaic symptom on the upper leaves. However, both of the viruses did not produce the same symptoms as naturally infected plants showed. Each virus also did not produce the color breaking syndrome on flowers. When the Mi-S and Mi-F were coinoculated to the stock seedlings, severe mosaic symptoms were observed on the leaves, and then these plants appeared color breakings of flower at about 10 weeks after inoculation. This result indicated that the color breaking syndrome

on stock might be caused by double infection of the Mi-S and Mi-F.

From these results, Mi-S and Mi-F isolated from *M. incana* were identified as CMV and TuMV, respectively. CaMV and TuMV have been isolated from *M. incana* (1) as well as from many ornamental plants. However, this is the first report for *M. incana* as a natural host of CMV.

요 약

1995년, 잎에 모자이크를 나타내고 꽃에 얼룩무늬증상을 보이는 스톡(*Matthiola incana*)을 대관령에서 채집하였다. 이병된 스톡으로부터 2종류의 바이러스를 분리하고, 기주범위, 혈청학 및 전자현미경 실험을 통하여 오이 모자이크 바이러스(CMV) 및 순무 모자이크 바이러스(TuMV)로 동정하였다. 분리한 CMV와 TuMV를 각각 어린 스톡에 접종하였을 때, 자연 발생된 병징은 발현되지 않았다. 그러나 이들 바이러스를 혼합하여 접종한 스톡에서는 잎에 심한 모자이크 증상과 함께 꽃에 얼룩무늬가 형성되었다.

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