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Bean Yellow Mosaic Virus and Cucumber Mosaic Virus Causing Mosaic Disease on Gladiolus in Korea

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그라디오러스에 발생하는 BYMV와 CMV에 관한 연구

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ABSTRACT

A mosaic disease of gladiolus has been commonly observed with an infection rate of 43.3% in the field. Bean Yellow Mosaic Virus(BYMV) produced veinal spreading lesions on *Cheonopodium amaranticolor*, veinal necrosis and severe leaf distortion on *Phaseolus vulgaris* 'Scotia' and mosaic on *Vicia faba*. Cucumber Mosaic Virus(CMV) produced local lesions on *C. amaranticolor*, mosaic symptoms on *Nicotiana glutinosa* and *Cucumis sativus*. BYMV and CMV were transmitted by the green peach aphid. Purified BYMV and CMV had a typical maximum absorption at 260nm. In agar gel diffusion test, BYMV and CMV gave positive reaction with their homologous antiserum. The size of BYMV was 750nm in length, and CMV was 30nm in diameter.

INTRODUCTION

A mosaic disease is distributed world wide in gladiolus. Berkeley[3] described that bean yellow mosaic virus(BYMV) and cucumber mosaic virus (CMV) were isolated from gladiolus by indicator reactions in 1953. In recent years, Zettler *et al.*[14] isolated BYMV from gladiolus in Florida, tomato spotted wilt virus was isolated from gladiolus by Lee *et al.*[8] in Australia.

CMV, BYMV, tobacco ringspot virus, tomato ringspot virus, tomato spotted wilt virus and aster yellows agents have all been reported in gladiolus. The virus diseases occurring in gladiolus were not reported in Korea. Therefore, in this paper, the host plant test, insect transmission, purification, serology, and electron microscopic studies were carried out for BYMV and CMV in gladiolus.

MATERIALS AND METHODS

Host plants test: Infected gladiolus were collected from the field of Horticulture Experimental Station in Suweon. Pieces of infected leaf material with prominent symptoms were ground up with a pestle and mortar in 0.01M phosphate buffer, pH 7.0. Leaves of indicator plants were rubbed with a sterilized piece of cotton with the inoculum. Caborundum(600 mesh) were dusted on leaves before inoculation and the leaves were washed with tap water soon after inoculation.

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Insect transmission: Green peach aphids(*Myzus persicae* Sulz.) were used in insect transmission studies using *Vicia faba* infected with BYMV, and *Nicotiana glutinosa* infected with CMV as virus source plants. Healthy *Pisum sativum* and *Vigna sesquipedalis* were fed for BYMV with 3 aphids per plant. After 24 hours, the aphids were killed by spraying insecticide. For the transmission of CMV, healthy *Cucumis sativus* was fed and the same method for BYMV was used.

Serological test: Agar gel diffusion test for BYMV was done according to the modified method of Fransisco[6]. The agar medium was composed of 0.08% of agar, 0.25% sodium dodecyl sulfate(SDS), and 1% sodium azide all in a 0.01M phosphate buffer, pH 7.3. The antigens were prepared by homogenizing 1g of infected tissues in a mortar and pestle with 2ml of 0.01M phosphate buffer, pH 7.3, and 3% of SDS, and also the purified virus was treated with 3% of SDS. The agar medium was poured into 9cm petri dishes and the wells were marked with an iron ring of 8mm in diameter. Then the petri dishes treated were incubated in a moist chamber at 25C. Cucumber mosaic virus and its antiserum were used in a serological test by the method of Lee *et al.*[9]

Purification: BYMV; The virus source for the purification experiments was leaves of BYMV infected *V. faba*. The plants were grown in green house. Infected *V. faba*. leaves were harvested 15 days after inoculation and chilled for one hour at 4C before purification. Tissues were homogenized for 1.5 min in a Waring blendor in 2 folds of chilled 0.1M citrate buffer, pH 7.3, containing 0.01M sodium diethyl dithiocarbamate. The homogenized materials were clarified using 8% of a 1 : 1 mixture of n-butanol and chloroform, stirred for 30 min, and centrifuged at 9,000 G for15 min, Supernatants were decanted and brought to 1.75% NaCl. Then 4%(W/V) of polyethylene glycol (PEG, M.W=6,000) was added and isolved for 60 min with magnetic stirrer and let stand one hour at 4C. The precipitate was recovered by low speed centrifugation at 8,000 G for 10 min. The virus was pelleted by high speed centrifugation at 110,000 G for 120 min. The pellets were resuspended in 0.1M phosphate buffer, pH 7.5. The virus was further purified by centrifugation at 48,000G for 180 min. using 40% of CsCl. The UV absorbing band was collected and dialized virus solution was centrifuged at 110,000 G for 120 min. The virus pellets were resuspended in 0.01M phosphate buffer, pH 7.5. CMV; Infected *Nicotiana tabacum* 'Ky-57' were homogenized in 0.5M citrate buffer, pH 6.5, containing 0.1M thioglicolic acid and 0.01M sodium ethy lenediamine tetraacetate. Chloroform was added to the materials and centrifuged at 9,000 G for 20 min. PEG, M.W=6,000, was added to the aqueous phase and the mixture stirred for one hour and dialized for 30 min in the refrigerator. The dializate was centrifuged at 9,000 G for 20 min. Pellets of precipitated virus were resuspended in 0.05 M sodium borate buffer, pH 8.5. The resuspended pellets were centrifuged at 105,000 G for two hours and 30 min. Pellets were resuspended in 0.01M borate buffer and left overnight in the refrigerator. The resuspended solution was clarified by centrifugation at 8,000 G for 15 min. Samples were layered on top of sucrose gradients(10~40%) and centrifuged at 48,000G for three hours. Then the virus bands were removed with a hypodermic syringe. The virus was pelleted by centrifugation at 105,000 G for two hours and thirty minutes and pellets were resuspended in 2ml of 0.01M borate buffer.

RESULTS

Investigation of virus disease: Mosaic disease symptoms on gladiolus were observed prevalently in the field. The investigated gladiolus cultivars were Hector, Snow Princess, Topase, and Fire Brand. The percentage of virus infection in gladiolus cultivars is shown in Table 1. The average infection was 43.3%.

Table 1. Incidence of gladiolus virus disease in the field.

Gladiolus cultivar	Number of plants observed	% of infection
Hector	100	61
Snow Princess	100	36
Topase	100	7o
Fire Brand	100	56
Average	100	43.3

Host range and symptomatology: Thirteen indicator plants were inoculated to investigate the host range of the viruses from gladiolus. The results are shown in Table 2. When BYMV from gladiolus was inoculated, veinal spreading lesions were produced on *Chenopodium amaranticolor*, and *C. quinoa*. *Phaseolus vulgaris* 'Scotia', and *P. vulgaris* 'Top crop' showed veinal necrosis and leaf distortion. Mosaic symptoms were produced on *Vigna unguiculata*, *V. sesquipedalis*, and *Vicia faba*, the inoculated leaf of *V. faba* produced large ringed local lesions. CMV from gladiolus produced severe mosaic symptoms on *Nicotiana glutinosa* and *N. tabaucum* 'B-Y', while *Cucumis sativus* showed a mild mosaic. Necrotic local lesions were produced on *Chenopodium amaranticolor*, *C. quinoa*, *V. sesquipedalis*, and *V. unguiculata*. In *Gomphrena globosa*, inoculated leaves and upper leaves all produced necrotic spot lesions.

Table 2. Reaction of indicator plants to mechanical inoculation of BYMV and CMV isolated from gladiolus.

Host species	Symptoms produced by	
	BYMV	CMV
CHENOPODIACEA		
Chenopodium quinoa Willd	Vs/Vs	L /–
C. amararanticolor Coste & Reyn	Vs/Vs	L /–
SOLANACEAE		
Nicotiana glutinosa	– / –	M/M
N. tabacum 'Ky-57'	– / –	M/M
N. tabacum 'Bright yellow'	– / –	M/M
EARACEAE		
Phaseolus vulgaris 'Scotia'	Vn/Vn	–/–
P. vulgaris 'Top crop'	Vn/Vn	–/–
Vigna unguiculata Savr	M / M	L /–
V. sesquipedalis Fruwirth	M / M	L /–
Vicia faba	Lr / M	L /–
CUCURBITACEAE		
Cucumis sativus	– / –	M/M
AMARANTACEAE		
Gomphrena globosa	– / –	L /L

* L: Local lesions, M: Mosaic symptoms, Vs: Veinal spreading lesions, Vn: Veinal necrosis, Lr: Ringed local lesions, –: non reaction.

Table 3. Transmission of two viruses from gladiolus by *Myzus persicae*.

Host tested	% of transmission	
	BYMV	CMV
Pisum sativum	66.6	–
Vigna sesquipedalis	64.6	–
Cucumis sativus	–	75.6

–:Not tested

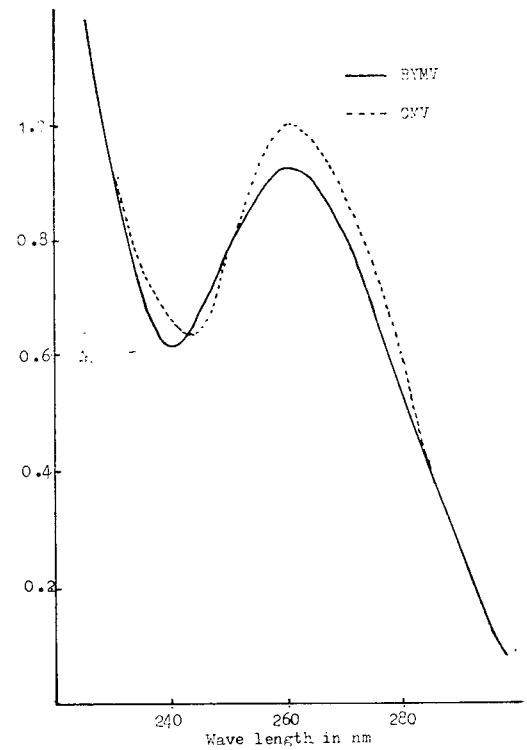

Fig. 1. Ultraviolet absorption spectrum of purified BYMV and CMV from gladiolus.

Insect transmission: The results, shown in Table 3., indicate that the BYMV and CMV from gladiolus were transmitted by *Myzus persicae*

Ultraviolet absorption: The typical absorption spectra of BYMV and CMV preparations are shown in Fig. 1. Maximum absorption of BYMV was 260nm and minimum absorption was at 240nm. CMV has a maximum absorption at 260nm and minimum absorption at 245nm.

Serology: Serological tests of BYMV and CMV were done using the agar gel diffusion test. BYMV reacted with BYMV antiserum(Plate 3). CMV also reacted with CMV antiserum(Plate 6). Those pricipi-

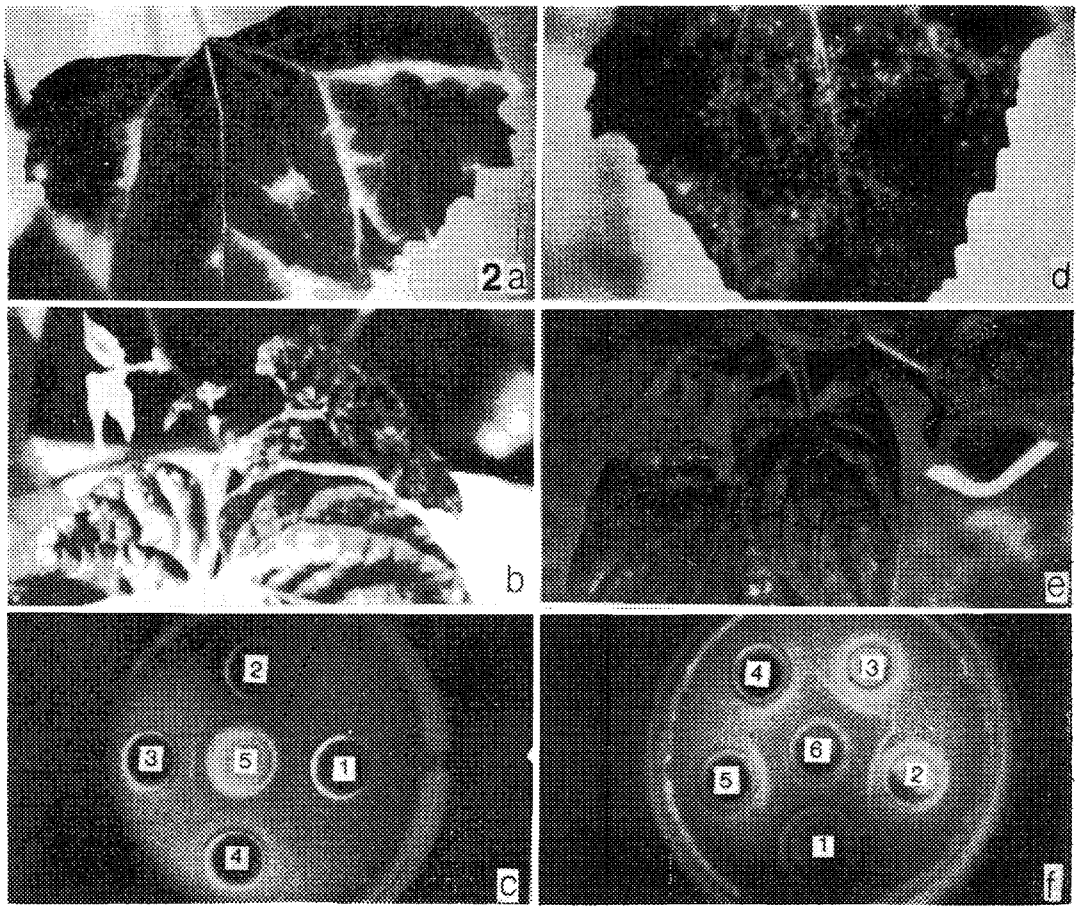

Fig. 2-(a to e). a) Vein-clearing on *Chenopodium amaranticolor* infected with BYMV. b) Leaves of *Phaseolus vulgaris* 'Scotia' infected with BYMV. c) Serology in agar gel diffusion test; Number 1 well contain fresh sap from *Vicia faba*, number 2 purified virus from gladiolus treated with SDS, number 3 sap frominfected *V. faba* treated with SDS, number 5 fresh sap from *V. faba*, and number 5 BYMV antiserum. d) Chlorotic local lesions on *Cheonopodium amaranticolor* infected with CMV. e) Mosaic symptoms on *Nicotiana glutinosa* infected with CMV. f) Serology in agar gel diffusion test; Number 1 well contain freash sap from *Cucumis sativus*, number 2 and 3 purified virus from gladiolus, number 4 and 5 sap of infected *N. tabacum* 'Ky-57' and number 6 CMV antiserum.

tin bands were well formed homologously for virus and tis antiserum.

Electron micorscopy: Electron micorgraphs of purified BYMV and CMV showed the typical shapes of each ivrus. BYMV was about 750nm in its most prevalent length. The size of CMV particles were about 30nm in diameter.

DISCUSSION

BYMV and CMV have been identified for the first time in Korea by studing the reaction in host plants, serology, insect transmission, and particle morphology.

The gladiolus isolate of BYMV produced veinal necrosis and severe leaf distortion symptoms on Scotia beans, but this is different from the reports of Lee *et al*[8], Alper *et al*[2], and Zettler *et al*[14]. On *C. amaranticolor*, the symptoms are not similar to the reports of Lee and Zettler, but the veinal spreading lesions produced by Iris-isolates of BYMV were similar. These diffierences and similarities are not supprising in view of the considerable variability of BYMV isolates in expression of host plant symptoms.

Except host range and symptomatology, our studies of serology, insect transmission and particle morphology were not different from the reports of Berkrley[3], Lee *et al* [10], and Zettler *et al*[14].

These results indicate that the gladiolus are host of BYMV and CMV. Coneivably, BYMV and CMV are prevalently distributed on gladiolus in Korea. Since BYMV from gladiolus were not show specific generally on the each host plants reaction, serology, insect transmission, and particle morphology.

Five viruses have been reported from gladiolus, but only BYMV and CMV were identified. In this study other viruses were isolated through the indicator plants test, but the viruses have not been identified. Therefore, the unidentified viruses should be studied further.

적 요

그라디오러스에 발생하는 바이러스병을 포장에서 조사한 결과 이병율이 43.3%이었다. BYMV는 명아주에 엽맥괴사병징, 강남콩에 심한 모자익 병징 그리고 잠두에 모자익 병징을 일으켰다. CMV는 명아주에 국부병반, 담배와 오이에 모자익 병징을 일으켰다. 분류동정된 두 바이러스는 모두 복숭아 혹진딧물에 의하여 전염 되었다. 바이러스를 순화하여 흡광도를 측정한 결과 두 바이러스 모두 260nm에서 최고의 흡광도를 나타내었다. 한천내확산법을 이용한 항혈청과의 반응결과 SDS를 처리한 BYMV와 CMV는 각각 그 바이러스의 항혈청과 뚜렷한 반응대를 형성하였다. 바이러스 입자의 전자현미경검경 과파 BYMV는 길이가 750nm였으며, CMV는 직경이 30nm이었다.

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