한국식물보호학회지 : 제20권 제 2 호
Korean J. Pl. Prot. 20(2) : 76~82(1981)

Isolation and Properties of Cucumber Mosaic Virus Inducing Mosaic Symptoms in *Hippeastrum hybridum* Hort.

J.S. Kim,* H.B. Kim,** and S.H. Lee*

아마리리스에 모자익병을 일으키는 CMV에 관한 연구

김 정 수*·김 홍 배**·이 순 형*

ABSTRACT

Cucumber Mosaic Virus (CMV) was isolated from naturally infected *Hippeastrum hybridum*. The virus caused mosaic symptoms on *Nicotiana glutinosa* and local lesions on *Vigna unguiculata*. The thermal inactivation point was 56C, dilution end point 10^{-3} and longevity in vitro was 2 days for CMV from Hippeastrum. Purified virus was obtained using citrate chloroform extraction procedure and polyethylene glycol precipitation followed by sucrose density gradient centrifugation. Purified virus had a typical absorption at 245nm. Electron micrographs of the purified virus from Hippeastrum showed spherical particles with 30nm in diameter. The purified virus reacted with CMV antiserum in agar gel double diffusion test.

INTRODUCTION

Cucumber Mosaic Virus (CMV) was originally described by Doolittle (1916) and Jagger (1916). The particle of CMV was first described by Sill, Burger and Walker (1952) through the methods of scientific identification.

CMV is world wide in distribution. The virus causing mosaic symptoms in *Cucumis sativus* had a wider host range and attacked a greater variety of vegetables, ornamentals and other plants than any other viruses. Price (1940) recorded 191 susceptible species in 40 families, Komuro (1973) reported that inoculated 39 families were infected with CMV, the Japanese common strain, and Chung, Park and Lee (1975) reported 71 species of 27 families were infected with CMV in Korea.

Cucumber mosaic affect plants by causing mottling or discoloration and distortion of leaves, flowers and fruits. Infected plants may be greatly reduced in size or they may be killed. In cucumber, the characteristic symptoms are a yellow mottle on all leaves developed after infection, some leaf distortion and stunting of the plant. The end of young fruit first become mottled with yellowish green and this gradually spreads over the entire fruit. In *N. tabacum*, pale green circular spots are produced on the inoculated leaves two or three days after inoculation, but no necrotic lesions are produced. Systemic infection first shows as a slight clearing of the veins and this is followed by a mild general mottle.

Chenopodium amaranticolor and *C. quinoa* are

* Dept. of Plant Pathology, Institute of Agricultural Science, Suweon, Korea.
** Dept. of Agriculture, Dongguk University, Seoul, Korea.

suitable for local lesion assay, and Sill and Walker (1953) reported primary leaves of cowpea as CMV assay plant from 12 to 15 days old gave the highest number of lesions and the number declined as the age of plant increased. Frank (1967) used *Gomphrena globosa* as a virus indicator because the plants produced easily distinguishable local lesions.

Several methods for purification of CMV have been known. Tomlinson, Shepherd and Walker (1959) using the Y strain of CMV described a purification procedure consisting of homogenization of infected tobacco leaves with phosphate buffer, and after centrifugation of the clarified extract at high speed, resuspended the virus in phosphate buffer (pH 7.5). They obtained spherical particles 40nm in diameter. Scott (1963) obtained CMV particles of 28~30nm in diameter by using dialysis of the aqueous phase against 0.005M borate buffer (pH 9.0) followed by 3 cycles of differential centrifugation.

Five viruses, Hippeastrum Mosaic virus (Brant and Vanden Heuvel, 1965), Tobacco Mosaic Virus (Deleeuw, 1972), CMV (Kahn and Smith, 1963), Tomato Spotted Wilt Virus (Smith, 1935), and Sunflower Mosaic Virus (Smith, 1957), have been reported in Hippeastrum plants.

The incidence of viral diseases in ornamentals has not been investigated in Korea. And also, there is a few reports to the identification of plant viruses (Chang, 1978). Especially, the mosaic disease in Hippeastrum did not detected.

Therefore, this study was carried out to know the host range, physical properties, purification, serology and morphology of virus particle of the isolated virus from *Hippeastrum hybridum*.

MATERIALS AND METHODS

Source of inoculum: Hippeastrum plants showing mosaic symptoms were collected in Jeju island, kept in the green house and used as inoculum sources.

Mechanical inoculation of host plants: Infected leaves showing mosaic symptoms were detached and homogenized in sodium phosphate buffer (pH 7.0) using mortar and pestle. Each test plant was inoculated with a piece of cotton dipped into the inoculum. Inoculated leaves were rinsed with tap water.

Purification: Infected *N. tabacum* 'ky-57' (340g) were homogenized in 0.5M sodium citrate buffer (pH 6.5) containing 0.1% thioglicolic acid and 0.01M sodium ethylendiamine tetraacetate. Chloroform was added to the supernatants to bring about 50% in concentration. The homonized materials were centrifuged at 8,000G for 20 minutes. Polyethylene glycol, M.W 6,000 (30g), was added to the aqueous phase and the mixture stirred for one hour and dialized for 30 minutes in refrigerator to increase the precipitation and concentration of virus (Clark and Lister, 1971, Shohara and Osaki, 1974). The dialyzate was centrifuged at 9,000G for 20 minutes. Pellets of precipitated virus were resuspended in 30m*l* of 0.005M sodium borate buffer (pH 8.5). The resuspended pellets were centrifuged at 95,000G for two hours and 30 minutes. Pellets were resuspended in 3.5m*l* of the borate buffer and left overnight in the refrigerator. The suspension was clarified by centrifugation at 6,000G for 15 minutes. Samples were layered on top of sucrose gradients (10~40%) and centrifuged at 70,000G for three hours. Then the virus bands were removed with a hypodermic syringe. The virus was pelleted by centrifugation at 98,000G for two hours and 30 minutes, and the pellets were resuspended in 2m*l* of borate buffer. The resuspended virus was centrifuged at 6,000G for 15 minutes. All procedures were carried out at 4C.

Serological test: Serological test was conducted using agar gel double diffusion method. Agar gel was prepared with 1% agar in 0.01M sodium phosphate buffer, pH 7.3, containing 0.0025M ethylen diamine tetraacetic acid disodium salt and 0.02% sodium azide as a preservative. CMV antiserum (homologous titer 1/1280) was obtained from the virology laboratory (Institute of Agriculture Sciences). Antigens used were purified virus preparation, CMV from cucumber and zinger which were obtained from the virology laboratory. Antigens were diluted 10 times in 0.005M borate buffer (pH 8.8) and antiserum was diluted 10

times in 0.85% NaCl.

RESULTS

Host range: Twenty seven plants were inoculated to test the host range of the virus from Hippeastrum (Table 1). Thirteen plants showed systemic mosaic symptoms. *Datura stramonium* and *Cucurbita pepo* showed systemic mosaic symptoms on inoculated leaves, but upper leaves were not infe-

Table 1. Reactions of host plants inoculated with the virus isolated from *Hippeastrum hybridum* by mechanical inoculation.

Plant species	Symptom ※	
	Inoculated leaves	Upper leaves
CHENOPODIACEAE		
Chenopodium amaranticolor Coste and Reyn.	L	—
C. quinoa Willd.	L	—
SOLANACEAE		
Nicotiana rustica L.	M	M
N. glutinosa L.	M	M
N. tabacum L. 'ky-57'	M	M
N. tabacum L. 'Bright yellow'	M	M
N. tabacum L. 'Samsun'	M	M
N. clevlandy L.	M	M
N. sylvestris L.	M	M
N. debneyii L.	M	M
Petunia hybrida Vilm	M	M
Datura metel L.	M	M
Lycopersicon esculentum Mill	—	—
Physalis floridana Rydberg.	M	M
CASSIACACEAE		
Cassia tora L.	—	—
EARACEAE		
Archis hypogaea L.	—	—
Phaseolus vulgaris L. 'Scotia'	—	—
Vigna unguiculata Savr.	L	—
Vicia faba L.	L	—
Vigna sesquepedalis Fruwirth.	L	—
CUCURBITACEAE		
Cucurbita pepo Mill	M	—
Cucumis melo L.	M	M
Citrullus battich Forsk	L	—
Cucumis sativus L.	M	M
AMARANTACEAE		
Gomphrena globosa L.	L	L
BRASSICACEAE		
Raphanus sativus L.	—	—
Brassica campestris L.	—	—

※; — Signified no symptoms, L signified local lesions and M signified mosaic symptoms.

cted as a result of reinoculation on *N. tabacum* 'ky-57'.

Seven plants produced local lesions without systemic symptoms. *Citrullus battich* Forsk showed local lesions on inoculated leaves, but when upper leaves were inoculated on *N. tabacum* 'ky-57', any symptoms were not showed. No symptoms were observed and no virus was recovered from six plants.

Physical properties: The crude sap of *N. tabacum* 'ky-57' was used to find out the stability of the virus from Hippeastrum. The thermal inactivation point was 55C, longevity in vitro at room temperature was 2 days and dilution end point was 10^{-3}.

Purification: Tow virus zones were obtained by sucrose density gradient centrifugation. The main zone was 20mm~30mm apart from the above of tube. The second zone was 20mm apart below the main zone (Plate, 3). These zones were associated with infectivity of the virus to cowpea.

Ultraviolet absorption: The ultraviolet absorption of the virus was measured with a Beckman Model 26 spectrophotometer. A typical absorption spectrum of the purified virus preparations is shown in Fig 1. Maximum absorption was at 260nm and minimum absorption was at 245nm. The average ratio of absorbance at 280/260 and at 260/245 were about 0.07 and 1.13, respectively.

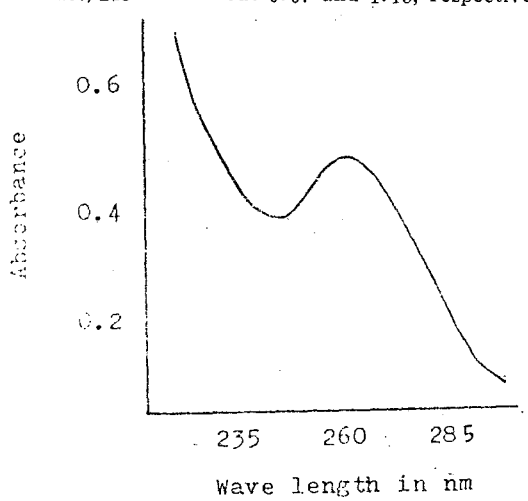


Fig. 1. Ultraviolet absorption spectrum of a purified preparation of CMV isolated from Hippeastrum

Serological test: Serological relationships of the virus from Hippeastrum were determined in agargel double diffusion test. All antigens reacted with CMV antiserum (Plate, 2).

Electron microscopy: Electron micrographs of the purified virus from Hippeastrum showed spherical particles, 30 nm in diameter (Plate, 4).

DISCUSSION

A virus inducing mosaic symptoms in Hippeastrum was proved to be CMV based on results of the symptoms in indicator hosts such as *C. sativus*, *V. unguiculata*, *C. amaranticolor*, *G. globosa*, *N. glutinosa*. The result of biological assay was similar to those of the reports of Chung, Park and Lee (1975), Lee and Chung (1978), Iwaki (1967) and Grogen, Hall and Kimble (1959).

R. sativus and *B. campestris* are hosts of CMV, but they were not infected by mechanical inoculations or insect transmissions. This result raised a possibility that it was caused by strain of virus or resistance of host plants to CMV (Chung, Park and Lee, 1975). When C. *melo* was infected with CMV, systemic mosaic symptoms were produced (Smith, 1972). The symptoms of C. *melo* in this host range test were similar to the reports of Chung and Smith. In *L. esculentum*, symptoms were not observed, but when it was infected with CMV, systemic mosaic (Chung, 1975) and necrotic local lesions (Iwaki 1967) were produced. The results were caused by the characteristic of the virus or resistance to CMV.

Generally, CMV was inactivated above 70C for 10 minutes, within a few days and some times in hours at room temperature (Gibbs, 1970). In this study, the results were similar to the reports of Yasuo (1973), and Tomlinson, Shepherd and Walker (1959), but generally, physical properties were not exactly same in accordance with the CMV strain.

Two virus zones were observed in sucrose density gradients centrifugation. The similar pattern in ultraviolet spectrum indicated that the virus of these zones had a same composition. The ultraviolet absorption pattern of the virus preparation

from Hippeastrum indicated that it was a typical RNA virus (Frowd and Tremaine, 1977, Kaper, Diener and Scott, 1965).

The virus purified from Hippeastrum reacted with CMV antiserum in agar gel double diffusion test and also antiserum with CMV isolated from zinger and cucumber. Finally, virus purified from Hippeastrum in Korea was similar to that of a CMV strain reported in Japan (So, 1980).

Electron micrscopy of CMV obtained by previous workers showed the virus as a spherical particle ranging from 35nm to 40nm in diameter (Tomlinson, Shepherd and walker 1959, Sill, 1952) An electron microscopy of purified CMV showed that the particles were spherical ranging from 28nm to 32nm in diameter (Lee and Chung, 1978, So, 1980). Electron micrographs of purified preparation of the virus in the present study also showed spherical particles of CMV and also the virus from Hippeastrum, 30nm in diameter.

Since CMV is transmitted by aphid species, the role of vector in epidemiology of mosaic disease in Hippeastrum must be studied in the future.

적 요

아마리리스에 모자익병을 일으키는 병원바이러스의 기주범위, 물리적성질, 혈청반응을 조사하고 바이러스 입자를 전자현미경으로 관찰을 하였다.

바이러스의 지표식물 검정결과 *C. amaranticolor*, *V. unguiculata*, *C. sativus* *G. globosa*등 21종의 CMV감수성식물에 병징이 나타났다.

바이러스의 내열성은 55C(10분간)이며 내희석성은 10^{-3}이고 내보존성은 실온에서 2일이었다.

한천내 확산법을 이용한 항혈청반응결과 뚜렷한 반응대가 형성되었으며, 바이러스흡광도는 260nm에서 최고였으며, 245nm에서 최저였다.

전자현미경에 의한 바이러스입자의 검경결과 직경이 30nm내외인 소구형 입자가 관찰되었다.

이상의 결과를 종합하면 아마리리스에 모자익병을 일으키는 것은 CMV에 의한 것으로 보인다.

REFERENCE

Chung, B.J., H.C. Park, and S.H. Lee. 1975. Studies on the host range of Cucumber Mosaic Virus in Korea. Korean J. Pl. Prot. 14 : 185-192.

Clark, M.F., and R.M. Lister. 1971. The application of polyethylene glycol solution concentration gradients in plant virus research. Virology 43 : 338-351.

Deleeuw, G.T.N. 1972. Tobacco Mosaic Virus in *Hippeastrum hybridum*. Neth. J. Pl. Path. 78 : 69-71.

Doolittle, S.P. 1916. A new infectious mosaic disease of cucumber. Phytopathology 6 : 145-147.

Franki, F.I. B. 1967. Effect of height intensities on spontaneous and virus induced local lesions in *Gomphrena globosa*. Phytopathology 57 : 329.

Frowd, M.A., and J.H. Tremaine. 1977. Physical, chemical and serological properties of Cymbidium Mosaic Virus. Phytopathology 67 : 43-49.

Gibbs, A.J., B.D. Harrison, and A.F. Murant. 1970. Description of plant viruses. CMI/AAB. No. 213.

Grogan, R.C., D.H. Hall, and K.A. Kimble. 1959. Cucurbit Mosaic Virus in california. Phytopathology 49 : 366-376.

Jagger, I.C. 1919. Experiments with the cucumber mosaic disease. Phytopathology 6 : 148-151.

Kaper, J.M., T.O. Diener, and H.A. Scott. 1965. Some physical and chemical properties of CMV (strain Y) and of its isolated ribonucleic acid. Virology 27 : 54-72.

Komuro, Y. 1973. Viruses of vegetables. 275-278.

Lee, S.H., K.W. Lee, and B.J. Chung. 1978. Purification and serology of Cucumber Mosaic Virus. Korean J. Pl. Prot. 17 : 29-31.

Mitsuro, I. 1967. Virus causing mosaic disease of Amaryllis in Japan. Ann. Phytopath. Soc. Japan. 33 : 237-243.

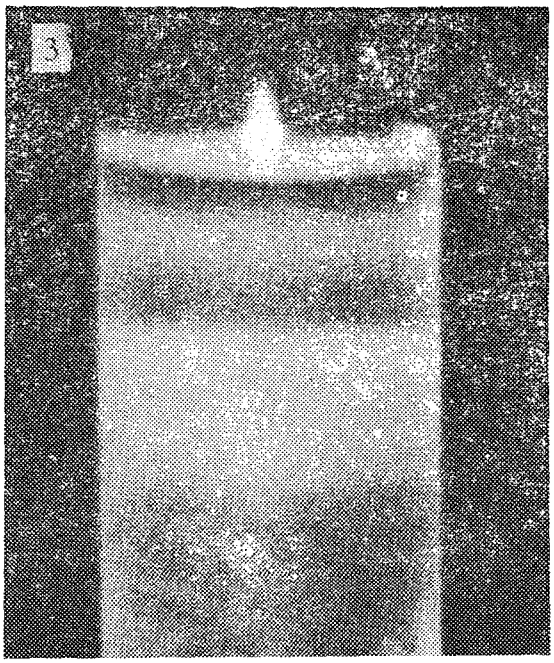
Price, W.C. 1934. Isolation and study of some yellow strain of cucumber mosaic. Phytopathology 24 : 743-761.

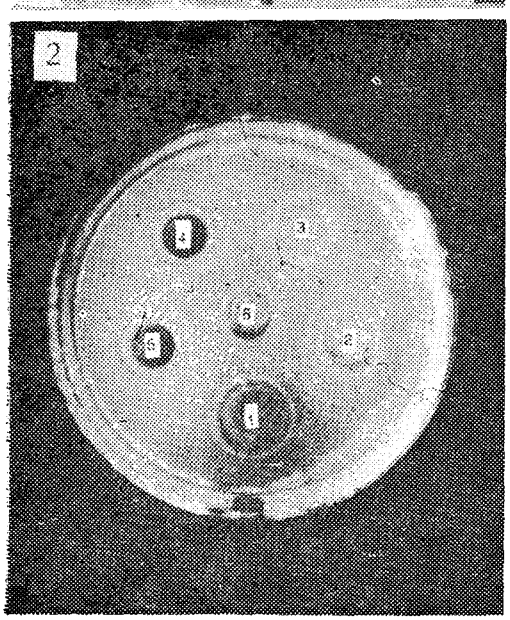
Scott, H.A. 1962. Purification of Cucumber Mosaic Virus. Virology 20 : 103-106.

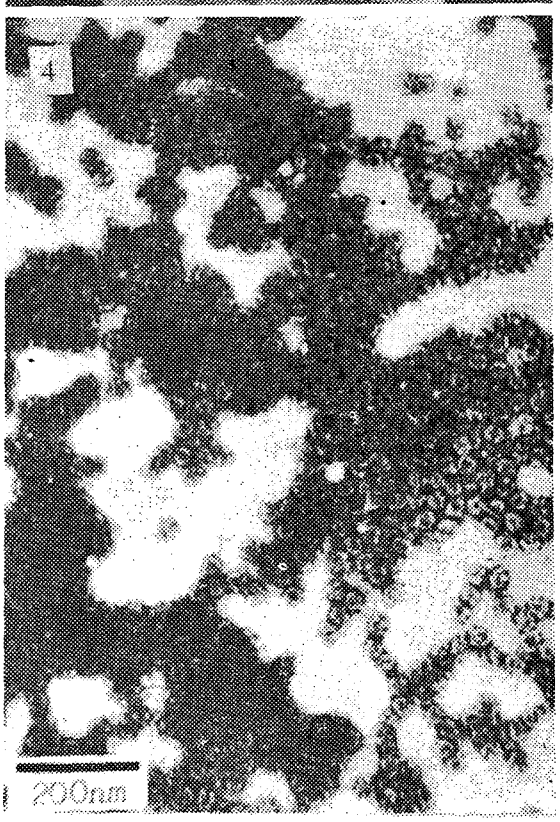
Sill, W.H.J., and J.C. Walker. 1952. Cowpea as assay host for cucumber virus 1. Phytopathology 42 : 328-330.

Sill, W.H.J., W.C. Burger., M.A. Stahman, and J.C. Walker. 1952. Electron microcopy of cucumber virus 1. Phytopathology 42 : 420-422.

Plate







Smith, K.M. 1972. A textbook of plant virus disease. Aca. Pre. 234-256.

So, I.Y. 1980. Studies on Ginger Mosaic Virus. Korean J. Pl. Prot. 19 : 67-72.

Takanami, Y., and K. Tomaru. 1976. Effect of EDTA on Cucumber Mosaic Virus and its application in purification. Virology 65 : 728-729.

Tomlinson, J.A., R.J. Shephered, and J.C. Walker. 1959. Purification, properties and serology of Cucumber Mosaic Virus. Phytopathology 49 : 293-299.

Legends of plate

1. Mosaic symptoms on *Hippeastrum hybridum* leaves infected with CMV.
2. Serology in agar gel diffusion test.
   1; fresh sap from healthy tobacco plants.
   2; purified virus from Hippeastrum.
   3; CMV from zinger.
   4; purified virus from Hippeastrum.
   5; CMV from cucumber.
   6; CMV-antiserum.
3. Seperation of CMV isolated from Hippeastrum by sucrose density gradients. The band contained infectious CMV.
4. Virus particles purified from infected Hippeastrum plants negatively stained with phosphotungstic acid (pH 6.8) by an Electron microscope (Hitachi, HU-11E) (X 60,000)