CUCUMBER MOSAIC VIRUS ISOLATED FROM WINGED BEAN (*PSOPHOCARPUS TETRAGONOLOBUS*) IN THAILAND AND FROM ASPARAGUS BEAN (*VIGNA SESQUIPEDALIS*) IN INDONESIA

Tsuneo TSUCHIZAKI[1], Mitsuro IWAKI[2], Norio IIZUKA[3], Nasir SALEH[4], Surapee KIRATIYA-ANGUL[5], and Nualchan DEEMA[5]

Abstract

Cucumber mosaic virus designated as CMV-M was isolated from winged bean showing ringspot on leaves in Thailand and from asparagus bean showing mosaic in Indonesia. The host ranges of CMV-M were wide, but the symptoms on most plants were mild or latent. CMV-M was transmitted by aphids in a non-persistent manner. Electron micrographs of partially purified preparations of CMV-M revealed the presence of isometric particles about 30 nm in diameter. CMV-M was closely related serologically to the yellow strain of CMV (CMV-Y). Prior infection of tobacco with CMV-M gave incomplete protection against systemic infection of CMV-Y or a strain of CMV isolated from tomato.

1. Introduction

Several viruses including cucumber mosaic virus (CMV) have been reported to occur naturally in winged bean (*Psophocarpus tetragonolobus*) and asparagus bean (*Vigna sesquipedalis*) (1, 2, 3, 4, 5). CMV is considered to be the world most prevalent virus in many plant species, mainly because the virus has a very wide host range and can be easily transmitted by many aphid species in a non-persistent manner. To our knowledge, CMV has not been reported previously in winged bean in Thailand and in asparagus bean in Indonesia. This report describes the virus from winged bean and asparagus bean which was identified as a mild strain of CMV (CMV-M).

2. Materials and methods

1) Source of the virus isolates The virus isolates used in this study were recovered from an infected winged bean plant in Thailand in 1980 and from an infected asparagus bean plant in Indonesia in 1982, and were designated as B-80-5 and C-82-5, respectively. The virus isolates were mechanically inoculated to tobacco (*Nicotiana tabacum*) and were maintained in plants. Infected leaves of tobacco were sources of inoculum for studies on host range, insect transmission, in vitro properties, purification and serology. Yellow strain of CMV (CMV-Y) and a strain of CMV isolated from tomato in Hokkaido (CMV-T) were obtained from K. Kiriyama, Cent. Res. Inst., Jap. Tobacco & Salt Public Corp., Yokohama, and T. Goto, Hokkaido Natl. Agric. Exp.

1) National Agriculture Research Center, Yatabe, Tsukuba 305, Japan.
2) National Institute of Agro-Environmental Sciences, Yatabe, Tsukuba 305, Japan.
3) Hokkaido National Agricultural Experiment Station, Sapporo 061-06, Japan.
4) Bogor Research Institute for Food Crops, Jalan Cimanggu Kecil 2, Bogor, Indonesia.
5) Department of Agriculture, Bangkhen, Bangkok 10900, Thailand.

Stn., Sapporo, respectively.

2) Mechanical inoculation Inoculations were performed by rubbing crude sap in 0.1 M phosphate buffer, pH 7.0, on leaves previously dusted with Carborundum. Infection or lack of infection on test cultivars and species was confirmed by lesion assay on *Chenopodium amaranticolor*.

3) Insect transmission *Myzus persicae* was tested as a possible vector. After a starvation period of 1-2 hr, the aphids were allowed to probe on infected tobacco plants for 1-5 min. They were then placed on non-infected tobacco plants for 2 hr prior to being sprayed with insecticides.

4) Virus purification Inoculated leaves of tobacco, harvested 8 days after inoculation, were extracted in 2 volumes of 0.5 M citrate buffer containing 0.1% thioglycolic acid, pH 7.3, in a blender. The extract was expressed through cheesecloth. After addition of chloroform to the extract to reach 20% (v/v), the extract-solvent mixture was shaken for 15 min, and the emulusion was broken by centrifugation at 9,000 g for 15 min. After addition of 8% polyethylene glycol 6000 (PEG) and 0.3 M sodium chloride to the supernatant fluid, it was stirred for 1 hr, and was centrifuged at 9,000 g for 15 min. The pellet was resuspended in 0.01 M borate buffer, pH 9.0, and clarified by centrifugation at 9,000 g for 10 min. This cycle of differential centrifugation using PEG was repeated twice. The preparation was then centrifuged at 105,000 g for 90 min. The pellet was resuspened in 0.01 M borate buffer, pH 9.0, and clarified by centrifugation at 9,000 g for 10 min. The resulting supernatant fluid was centrifuged at 60,000 g for 3 hr in linear sucrose density gradient columns of 10 to 50% sucrose in 0.05 M borate buffer, pH 9.0. Following centrifugation, the gradient columns were scanned at 254 nm and fractionated with an ISCO density-gradient fractionator. Ultraviolet-absorbing zone was collected and centrifuged at 105,000 g for 90 min. Final virus preparations were resuspended in distilled water.

5) Serology Ouchterlony double-diffusion tests were conducted in 0.75% agar with 0.85% sodium chloride and 0.05% sodium azide. Antiserum against CMV-Y was provided by K. Kiriyama.

6) Electron microscopy For electron microscopic observation, partially purified virus was fixed for 10 min in neutralized formaldehyde and then stained with 1% phosphotungstic acid at pH 7.0.

7) Corss protection Cross protection was investigated in tobacco plants by inoculating lower leaves with B-80-5 or C-82-5, follwed by subsequent inoculation of the upper leaves with a challenge virus 20 days later. Healthy tobacco plants of comparable age were inoculated with the challenge virus at the time of challenge incoulation.

3. Results

1) Host range

Of the 21 species of plants inoculated mechanically with B-80-5 or C-82-5, 18 species

Table 1. Host range of two isolates of cucumber mosaic virus from winged bean (B-80-5) and asparagus bean (C-82-5).

Hosts	Virus isolates	
	B-80-5	C-82-5
Legumes		
Glycine max	—[a)]	—
Phaseolus angularis 'Akatsuki Dainagon'	CS	M
'Sakae'	LS	
P. vulgaris 'Kairyootebo'	LS	LS
'Honkintoki'	SN	SN, M
Pisum sativum	—	—
Psophocarpus tetragonolobus	CS	CS
Vigna radiata		L
V. sesquipedalia 'Kurodanesanjaku'	mM	mM
'Akadanesanjaku'		CS
V. sinensis	LS	mM
Nonlegumes		
Brassica campestris	—	—
Chenopodium amaranticolor	L	L
C. quinoa	L	L
Cucumis sativum	LL	LL
Cucurbita moschata	CS	CS
Gomphrena globosa		LS
Lycopersicon esculentum		LS
Nicotiana glutinosa	mM	
N. tabacum 'Sumsun NN'	LS	L, LS
'Blight Yellow'	LS	
'Xanthi'		LS
Petunia hybrida	mM	LS
Physalis floridana	LS	LS
Tetragonia expansa	L, CS	
Zea mays	LS	M

a) L: necrotic or chlorotic local lesions, LL: symptomless local infection, M: mosaic, mM: mild mosaic, CS: chlorotic spot, SN: stem necrosis, LS: symptomless systemic infection, —: no infection.

developed local or systemic symptoms, or latent infection (Table 1). The host ranges of both isolates were wide, but the symptoms on most plants were mild or latent. *Chenopodium amaranticolor* was found to be an excellent local lesion host.

2) Insect transmission

B-80-5 was transmissible in a non-persistent manner by *Myzus persicae* (1 infected plant per 10 plants exposed), but C-82-5 was not transmissible by *Aphis craccivora* (0/8).

3) Properties in vitro

Crude sap from B-80-5 infected tobacco leaves was infectious after 10 min at 50°C but not at 60°C, and after a dilution of 10^{-3} but not 10^{-4}. It was also infectious for 1 day at room temperature but not after 4 days. In the same test, C-82-5 was inactivated after 10 min at 55–65°C, had a dilution end point ranging between 10^{-3} and 10^{-4}, and was infectious after 7 days at room temperature.

4) Electron microscopy

Under the electron microscope, partially purified preparations of B-80-5 and C-82-5 appeared to consist of isometric particles with a diameter of about 30 nm.

5) Serology

Antiserum to CMV-Y gave reactions of homology between CMV-Y antigen and B-80-5 or C-82-5 antigen in double-diffusion test with agar. This result showed that B-80-5 and C-82-5 were closely related serologically to CMV-Y.

6) Cross protection

Prior infection of tobacco plants with B-80-5 or C-82-5 gave incomplete protection against systemic infection of CMV-Y or CMV-T. B-80-5 reduced the transmission of CMV-Y by 16%, and C-82-5 reduced the transmission of CMV-T by 83%. Non inoculated controls failed to show any symptoms, and all the tobacco plants inoculated with a challenge virus showed only systemic symptoms produced by the challenge virus.

4. Discussion

The isometric virus isolates recovered from winged bean in Thailand (B-80-5) and asparagus bean in Indonesia (C-82-5) were identified as a mild strain of cucumber mosaic virus (CMV-M) by host range and symptomatology, particle morphology, and their close serological relationships to Y strain of CMV. Both B-80-5 and C-82-5 produced similar infection patterns on many species, and from this point, B-80-5 and C-82-5 could be considered to be same strain.

B-80-5 induced symptoms in winged bean that resembled closely those described for a strain of CMV designated as CMV-WB in winged bean in Florida (3). However, B-80-5 differed from CMV-WB in that either B-80-5 failed to induce symptoms in many species, or B-80-5 induced symptoms were less severe than those induced by CMV-WB.

A strain of CMV was reported to occur naturally in cowpea in Florida (1). C-82-5 resembled the cowpea strain of CMV in symptomatology among herbaceous hosts. Both differed from most of the reported strains of CMV in causing systemic disease in cowpea and asparagus bean plants, and in remaining essentially latent in tobacco plants.

This is the first report of CMV occuring naturally on winged bean in Thailand and on asparagus bean in Indonesia.

Acknowledgments

We thank K. Kiriyama, Cent. Res. Inst., Jap. Tobacco & Salt Public Corp., Yokohama for providing antiserum against CMV-Y, and T. Goto, Hokkaido Natl. Agric. Exp. Stn., Sapporo for providing CMV.

Literature cited

1. Anderson, C. W. (1955). Vigna and crotalaria virus in Florida. Plant Dis. Rept. 39: 345-357.
2. Fortuner, R., Fauquet, C., and Lourd, M. (1979). Diseases of the winged bean in Ivory Coast. Plant Dis. Rept. 63: 194-199.
3. Kuwite, C. A. and Purcifull, D. E. (1982). Some properties of a cucumber mosaic virus strain isolated from winged bean in Florida. Plant Dis. 66: 1071-1073.
4. Talens, L., and Talens, A.C. (1979). Identity of a strain of cowpea mosaic virus in winged bean (*Psophocarpus tetragonolobus*). Philipp. Phytopathol. 15: 62-68.
5. Tsuchizaki, T., Yora K., and Asuyama, H. (1970). The viruses causing mosaic of cowpeas and azuki beans, and their transmissibility through seeds. Ann. Phytopath. Soc. Japan 36: 112-120.