BLACKEYE COWPEA MOSAIC VIRUS FROM ASPARAGUS BEAN (*VIGNA SESQUIPEDALIS*) IN THAILAND AND MALAYSIA[1)]

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Abstract

A sap-transmissible virus isolated from infected asparagus bean (*Vigna sesquipedalis*) in Thailand and Malaysia had a relatively wide host range, but the plants showing systemic symptoms were limited to Leguminosae. In asparagus bean juice, infectivity was lost by heating at 55-65°C for 10 minutes, by diluting to 10^{-4}-10^{-5}, and by aging at 20°C for 1-3 days. The virus consisted of flexuous filaments about 750 nm in length, and was transmitted by aphids in a non-persistent manner. The isolate from Thailand was seed-borne in cowpea but not in asparagus bean. In double-diffusion tests in agar gel plates containing lithium 3,5-diiodosalicylate, the virus reacted strongly with antiserum to the Florida isolate of blackeye cowpea mosaic virus (BlCMV) and bean common mosaic virus (BCMV). Based on these results, the virus was identified as BlCMV.

1. Introduction

Blackeye cowpea mosaic virus (BlCMV) and cowpea aphid-borne mosaic virus (CAMV) are two potyviruses pathogenic to cowpea (*Vigna sinensis*) and asparagus bean (*V. sesquipedalis*). BlCMV was first reported in the United States by Anderson (1955) (1). CAMV was reported in Italy by Lovisolo and Conti (1966) (7), and other worker have viral isolates having similar properties to those of CAMV from several parts of the world (2, 4, 5, 10).

A seed-transmissible virus disease of asparagus bean in Thailand was described by Deema (1976) (3). The virus consisted of flexuous filaments about 750 nm long and was transmitted by aphids in a non-persistent manner. The virus, however, was not identified.

This paper reports the identification and characterization of the BlCMV isolated from asparagus bean in Thailand and Malaysia.

2. Materials and methods

1) Source of the virus isolates and antisera The BlCMV used in this study was isolated from naturally infected, field-grown asparagus beans in Thailand in 1980 and in Malaysia in 1982. The isolates were designated as BlCMV-T and BlCMV-M, respectively. Some antisera were also used for the comparison of the serological relationships. The source of each antiserum was as follows: Florida isolate of BlCMV

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(BlCMV-Fla 2) and Morocco isolate of CAMV (CAMV-Mor) (D. Gonsalves, Cornell University, Geneva, USA); azuki bean mosaic virus (AzMV) (N. Iizuka, Hokkaido Natl. Agric. Exp. Stn. Sapporo); potato virus Y (PVY) (Y. Saito, Natl. Inst. Agro-Envir. Sci., Tsukuba); bean common mosaic virus (BCMV) and soybean mosaic virus (SMV) (T. Tsuchizaki).

2) Mechanical inoculation Sap inoculation was performed by rubbing the Carborundum-dusted leaves of test plants with a piece of cotton soaked in a homogenate of infected leaves prepared in 0.1 M phosphate buffer, pH 7.0.

3) Transmission Seeds from mechanically inoculated plants in the greenhouse were sown and raised in sterilized soil in pots in the greenhouse. The seedlings were checked for symptoms of BlCMV infection. Aphid transmission tests were carried out by using virus-free aphid, *Aphis craccivora* Koch, reared on healthy asparagus beans. Five aphids were used per test plant and each aphid was allowed 3-5 min of acquisition access period on a diseased plant with 1-2 hr preacquisition starvation, followed by an overnight inoculation access period on a healthy test plant.

4) Stability of virus sap For the study of the physical properties of the virus, leaves of asparagus bean with systemic infection were macerated in a mortar with a pestle. The crude sap was expressed through cheesecloth pads, and a 1:10 dilution was prepared for each isolate with 0.1 M phosphate buffer, pH 7.0. The diluted sap was divided into three parts for the study of the thermal inactivation points, dilution end point, and longevity of the virus in vitro.

5) Virus purification BlCMV-T was purified by the procedure of Tsuchizaki *et al.* (11) from infected leaves of asparagus bean plants 2-3 weeks after inoculation. The purified samples were provided for rabbit immunization and electron microscopy.

6) Electron microscopy For electron microscopic observation, small pieces of infected asparagus bean or bean leaves were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 4 hr at 4°C and post-fixed in 1% osmium tetroxide in the same buffer for 3 hr at 4°C. After dehydration with an acetone series, they were embedded in Spurr resin. The sections were stained with uranyl acetate and lead citrate.

7) Serology Antiserum against the virus was produced in a rabbit by three intramuscular injections of purified virus emulsified with Freund's complete adjuvant (1:1, v/v) at 3 week intervals. Antiserum was collected from the rabbit 4 weeks after the final injection. Ouchterlony double-diffusion tests were conducted in 0.8% agar with 0.85% sodium chloride, 0.5% lithium 3,5-diiodosalicylate, and 0.1% sodium azide.

3. Results

1) Host range studies

Table 1 shows the host range of BlCMV-T and BlCMV-M. The plants showing systemic symptoms were limited to the Leguminosae. Both BlCMV-T and BlCMV-M caused systemic symptoms on asparagus bean, azuki bean, bean and cowpea, and local

Table 1. Host range of two isolates of blackeye cowpea mosaic virus (BlCMV) from Thailand and Malaysia

Plant species	Isolates	
	BlCMV-T[a)]	BlCMV-M[a)]
Legumes		
Glycine max 'Yuzuru'	LS[b)]	—
Phaseolus angularis 'Wasedairyu No. 1'	M	M
'Kyoto Dainagon'	—	—
P. vulgaris 'Honkintoki'	M	VC, Y
'Kairyootebo'	L	LL
'Top Crop'	—	—
Pisum sativum	LL	—
Vicia faba	CS	—
Vigna sesquipedalis 'Kurodanesanjaku'	VB, M	LS
'Akadanesanjaku'	VB, M	M
V. sinensis 'Blackeye'	M	CS, M
V. radiata	M	LL
Nonlegumes		
Nicotiana glutinosa	—	—
N. tabacum 'Samsun NN'	LL	LL
Petunia hybrida	LL	—
Cucumis sativus	—	—
Chenopodium amaranticolor	L	L
C. quinoa	L	L
Gomphrena globosa	LL	LL
Zea mays	—	—

a) BlCMV-T and BlCMV-M are the Thailand and Malaysia isolates of BlCMV, respectively.
b) CS: chlorotic spot, L: local lesion, LL: symptomless local infection, LS: symptomless systemic infection, M: mosaic, VB: vein banding, VC: vein clearing, —: no infection.

lesions on inoculated leaves of *Chenopodium amaranticolor* and *C. quinoa*.

2) Transmission

Aphis craccivora was able to transmit BlCMV-T and BlCMV-M in a non-persistent manner.

Two out of 109 seedlings grown from seeds collected from blackeye cowpea plants infected with BlCMV-T in the greenhouse showed mosaic symptoms indicating the seed-transmissibility of the virus. However, all the seedlings grown from 109 seeds of Kurodanesanjaku asparagus bean plants infected with BlCMV-T in the greenhouse were healthy.

3) Stability in sap

BlCMV-T and BlCMV-M had similar properties in vitro. Infectivity of crude sap of leaves infected with BlCMV was lost by heating at 55-65°C for 10 min, by dilution to 10^{-4}-10^{-5}, and by aging at 20°C for 1-3 days.

4) Electron microscopy

Electron microscopic observation of purified preparations of BlCMV-T or leaf-dip

preparations from BlCMV-M infected plants stained with 1% phosphotungstic acid revealed the presence of flexuous rods about 750 nm in length. Both BlCMV-T and BlCMV-M induced pinwheel and circular inclusion bodies in various areas of the cytoplasm of the infected cells.

5) Serological tests

An antiserum prepared against the purified preparation of BlCMV-T had a dilution end point of 1/640 in ring interface precipitin tests with its homologous virus. In double-diffusion test in agar gel containing 0.5% lithium 3,5-diiodosalicylate, antisera to BlCMV-T, BlCMV-Fla 2, BCMV and AzMV reacted strongly with BlCMV-T and BlCMV-M. Each precipitin line to BlCMV-T and BlCMV-M coalesced. SMV antiserum reacted weakly with BlCMV-T and BlCMV-M. No reaction to BlCMV-T and BlCMV-M antigens was detected when antisera to CAMV-Mor and PVY were used (Table 2).

Table 2. Serological reaction of blackeye cowpea mosaic virus (BlCMV) to antisera of several potyviruses in agar gel diffusion plates containing 0.5% lithium 3,5-diiodosalicylate.

Antisera[a)]	Antigens[a)]	
	BlCMV-T	BlCMV-M
BlCMV-T	+	+
BlCMV-Fla 2	+	+
CAMV-Mor	-	-
BCMV	+	+
AzMV	+	+
SMV	(+)	(+)
PVY	-	-

a) Viral isolates and antisera used: BlCMV-T, BlCMV-M and BlCMV-Fla 2 are the Thailand, Malaysia and Florida isolates of BlCMV, respectively. CAMV-Mor: Morocco isolate of CAMV, BCMV: bean common mosaic virus, AzMV: azuki bean mosaic virus, SMV: soybean mosaic virus, and PVY: potato virus Y.
b) +: strong reaction, (+): weak reaction, -: no reaction.

4. Discussion

This study demonstrated that the Thailand and Malaysia isolates of BlCMV reacted strongly with antisera to BlCMV-Fla 2 and BCMV, but did not react with antiserum to CAMV-Mor. Taiwo and Gonsalves (8) reported that in reciprocal SDS-immunodiffusion tests, antisera to BlCMV and BCMV gave reactions of homology to BlCMV, and gave a weak reaction to CAMV. Results from our studies on particle size and morphology, cytoplasmic inclusions, host range and symptomatology, transmission and the serological relationships of the two isolates from Thailand and Malaysia clearly indicated that the isolates were BlCMV. This is the first report on the occurrence of BlCMV in Malaysia and Thailand.

The classification of BlCMV and CAMV was in a state of confusion until recently, when Lima *et al.* (6) suggested that BlCMV and CAMV should be regarded as distinct members of the potyvirus group. Taiwo *et al.* (9) reported that based on host range and serological similarities, some viral isolates previously assumed to be CAMV were actually BlCMV. In the preliminary report (12), we described that BlCMV-T was identified as CAMV. The results of this study, however, have demonstrated that BlCMV-T reacted strongly with antisera to BlCMV-Fla 2 and BCMV but did not react with antiserum to CAMV-Mor. Based on serological similarities, BlCMV-T should be regarded as BlCMV instead of CAMV.

The Thailand isolate of BlCMV was transmissible through cowpea seed but not through asparagus bean seed. It was reported that the rate of seed transmission of BlCMV depended on species or cultivars of hosts and virus strains (10). The BlCMV isolates collected from Thailand may be transmissible through seeds of other cultivars of asparagus bean.

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