PARTIAL CHARACTERIZATION AND SEROLOGICAL RELATIONSHIPS OF THREE POTYVIRUSES ISOLATED FROM LEGUMINOUS CROPS IN THAILAND

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ABSTRACT

Four isolates of soybean mosaic virus (SMV) from soybeans in Thailand were identified by host range, serology, transmission and electron microscopy. The host range of the four isolates differed in reactions in a few plants. Isolates of SMV from Thailand could be classified into two serological groups which were different from a SMV isolate from Japan. Two isolates of bean common mosaic virus (BCMV) from mungbeans in Thailand were transmitted by aphids and through mungbean seeds. The plants which showed systemic symptoms by mechanical inoculation of the isolates were limited to Leguminosae. Isolates of BCMV from Thailand consisted of flexuous filaments about 750 nm in length, which reacted strongly with antiserum to BCMV isolated from bean in Japan. A seed-borne, aphid-transmitted virus isolated from infected asparagus bean in Thailand had a relatively wide host range. The virus consisted of flexuous filaments about 750 nm in length, which reacted strongly with antiserum to the Florida isolate of blackeye cowpea mosaic virus (BlCMV). Based on these results the virus was identified as BlCMV. In double-diffusion test in agar gel containing 0.5% lithium 3,5-diiodosalicylate, BCMV and BlCMV were serologically identical, and SMV was serologically related to, but distinct from BCMV and BlCMV.

Introduction

Leguminous crops are very important as a source of protein in tropical regions. Virus diseases are considered to be a major limiting factor affecting the production of leguminous crops. The most prevalent viruses in leguminous crops may be the potyviruses, mainly because these viruses are seed-borne and can be easily transmitted by many aphid species in a non persistent manner. Deema (1976) reported that soybean mosaic virus occurred naturally in soybeans in Thailand. However, the virus has not been studied in detail and very little is known about its properties. This paper reports the partial characterization and serological relationships of soybean mosaic virus, bean common mosaic virus and blackeye cowpea mosaic virus isolated from soybean, mungbean and asparagus bean, respectively in Thailand.

Soybean mosaic virus (SMV) isolated from soybean (*Glycine max*)

We used four isolates, SMV-12B, SMV-27, SMV-43 and SMV-124, obtained from naturally infected, field-grown soybeans in various areas of Thailand in 1979.

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Table 1 Host reactions to infection with the four isolates of soybean mosaic virus from Thailand

Plant species	Soybean mosaic virus isolates[a]			
	SMV-12B	SMV-27	SMV-43	SMV-124
Chenopodium amaranticolor	—	L	—	—
C. quinoa	—	L	—	LL
Spinacia oleracea	—	LL	—	LL
Gomphrena globosa	LL			LL
Tetragonia expansa	—	L	—	LL
Brassica rapa	—	—	—	—
Arachis hypogaea	LL			—
Astragalus sinicus	LL			
Glycine max 'Shirotsurunoko'	CS, M	L, CS, M	CS, M	CS, M
'Okuharawase'	CS, M	CS	L, CS	CS, M
'Izuru'		L, M	CS	
Lathyrus odoratus	—			
Lupinas lutens	LL			
Medicago sativa	—			
Phaseolus angularis	LL	M	LS	LS
P. vulgaris 'Tsurunashi Kintoki'	M	L, CS	L, CS, M	M
'Top Crop'	L	—	—	—
'Yamashiro Kurosando'	—			LL
Pisum sativum	LL	—	—	LL
Trifolium pratense	—	—	—	—
T. repens	—	—	—	—
Vicia faba	LL	—	LL	—
Vigna mungo	—	—	—	—
V. radiata	LL	—	—	LL
V. sesquipedalis	—	—		—
V. sinensis	—	LS	—	LS
Datura stramonium	—			LL
Lycopersicon esculentum	—			—
Nicotiana clevelandii	LS			LS
N. glutinosa	—	—	—	—
N. tabacum 'Bright Yellow'	—	—	—	—
Petunia hybrida	—	—	—	LL
Sesamum indicum	LS	—	—	—
Cucumis sativus	—	—		
Zinnia elegans	—	—	—	—

* L : necrotic or chlorotic local lesions, M : systemic mosaic, CS : systemic chlorotic spot, LL : latent local infection, LS : latent systemic infection, — : no infection.

1 Host range

Reactions of the tested plants to infection by the four isolates of SMV are summarized in Table 1. *Glycine max* and *Phaseolus vulgaris* 'Tsurunashi Kintoki' were susceptible to all of the four SMV isolates and showed systemic symptoms. Local lesions developed on inoculated leaves of *Tetragonia expansa, Chenopodium amaranticolor* and *C. quinoa* after inoculation with SMV-27, and on *P. vulgaris* 'Top Crop' inoculated with SMV-12B.

2 Virus transmission

Aphis glycines, Myzus persicae, and *Aulacorthum solani* were able to transmit SMV in a non persistent manner. All seedlings grown from seeds collected from three cultivars of soybean plants inoculated with SMV-27 or SMV-43 in the greenhouse were healthy. However, one out of 179 seedlings grown from Okuharawase soybean plants infected with SMV-12B in the greenhouse became infected with the virus.

3 Purification and particle morphology

The purification procedure was as follows: homogenizing infected soybean leaves in 0.5 M citrate buffer containing 1% 2-mercaptoethanol, clarification with carbon tetrachloride, polyethylene glycol precipitation, and centrifugation in linear density gradient columns of 10–40% sucrose in 0.5 M phosphate buffer containing 0.01 M magnesium chloride. Electron microscopic examination of the purified preparation showed flexuous rods about 750 nm in length.

4 Serological tests

Antiserum against SMV-27 or SMV-12B had a dilution end point of 1 : 512 in ring interface precipitin test. Serological relationships among the SMV isolates were tested in agar gel diffusion plates containing 0.5% lithium 3,5-diiodosalicylate using antisera to SMV-12B, SMV-27 and SMV-H (an isolate of SMV from Japan). With the antiserum to SMV-27, SMV-27 spurred over SMV-12B and SMV-H, but not over SMV-124. SMV-124 formed lines of identity with SMV-27. With the antiserum to SMV-12B, SMV-12B spurred over SMV-27, SMV-124 and SMV-H. With the antiserum to SMV-H, SMV-H spurred over SMV-27, SMV-124 and SMV-12B (Table 2). These results indicate that SMV-12B, SMV-27 and SMV-H were serologically related but not identical; SMV-27 and SMV-124 were serologically undistringuishable.

Table 2 Serological reaction among isolates of soybean mosaic virus (SMV) in agar gel diffusion plates containing 0.5% lithium 3,5-diiodosalicylate

Antisera	Virus isolates			
	SMV-H	SMV-27	SMV-12B	SMV-124
SMV-H	+	+*	+*	+*
SMV-27	+*	+	+*	+
SMV-12B	+*	+*	+	+*

* Spur reaction was observed with the virus isolate homologous to the antiserum used.

Results from our studies on particle size and morphology, host range and symptomatology, transmission and serological relationships of the four virus isolates from Thailand clearly indicated that these viruses are SMV. SMV isolates from various locations in Thailand could be classified into two serotypes which were serologically different from a SMV isolate from Japan.

Bean common mosaic virus (BCMV) isolated from mungbean (*Vigna radiata*)

Two isolates of BCMV, BCMV-8 and BCMV-12, used in this study were recovered from naturally infected field-grown mungbeans in Thailand in 1979.

1 Host range

With the exception of three species of *Chenopodium, Tetragonia expansa* and *Sesamum indicum,* the host ranges of BCMV-8 and BCMV-12 were limited to the leguminosae. Both of the isolates produced systemic symptoms in bean, azuki bean, blackeye cowpea, *Vigna mungo* and mungbean. BCMV-8 and BCMV-12 differed slightly in their host range and symptomatology.

2 Seed transmission

Seed transmission of BCMV-8 and BCMV-12 in several species infected as seedlings was tested. BCMV-8 and BCMV-12 were transmitted in 4.1% (7/172) and 7.2% (18/248) of the mungbean (cv. 'M7A') seeds respectively. BCMV-12 was also found to be seed-borne at the rate of 1.3% (1/77) in bean (cv. 'Honkintoki').

3 Insect transmission

BCMV-8 and BCMV-12 were transmitted from bean by *Myzus persicae* to bean in a non persistent manner, and BCMV-12 was also transmitted by *Aphis craccivora. Aulacorthum solani* was able to transmit BCMV-8 but not BCMV-12.

4 Purification and particle morphology

BCMV was purified by the same procedure as that applied to SMV. Electron microscopic examination of the purified preparation showed flexuous rods of approximately 750 nm in length.

5 Serology

Antiserum against BCMV-12 had a dilution end point of 1:512 in ring interface precipitin test. In double-diffusion tests with agar containing 0.5% lithium 3,5-diiodosalicylate using antisera against BCMV-12 and BCMV isolated from bean in Japan (BCMV-J), the antigenic relatedness of BCMV-8, BCMV-12 and BCMV-J was serologically identical.

The virus recovered from mungbean in Thailand was identified as BCMV on the basis of particle size and morphology, host range and symptomatology, transmission and serology. Kaiser and Mossahebi (1974) described a strain of BCMV (M-BCMV) from mungbean in Iran. Our results with BCMV-8 and BCMV-12 were in general agreement with those of M-BCMV.

Blackeye cowpea mosaic virus (BlCMV) isolated from asparagus bean (*Vigna sesquipedalis*)

BlCMV used in this study (BlCMV-5) was isolated from a naturally infected asparagus bean plant in Thailand in 1980.

1 Host range

The plants showing systemic symptoms were limited to the Leguminosae. BlCMV-5 caused systemic symptoms in asparagus bean, azuki bean, bean and cowpea, and local lesions on inoculated leaves of *Chenopodium amaranticolor* and *C. quinoa.*

2 Transmission

Aphis craccivora was able to transmit BlCMV-5 in a non persistent manner. Two out of 109 seedlings grown from seeds collected from blackeye cowpea plants infected with BlCMV-5 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-5 in the greenhouse were healthy.

3 Purification and particle morphology

BlCMV-5 was purified by the same procedure as that applied to SMV. Electron microscopic

examination of the purified preparation showed flexuous rods of about 750 nm in length.

4 Serology

An antiserum prepared against the purified preparation of BlCMV-5had a dilution end point of 1/640 in ring interface precipitin tests with the homologous virus. In double-diffusion test in agar gel containing 0.5% lithium 3,5-diiodosalicylate, antiserum against the BlCMV-Fla 2 (Florida isolate of BlCMV from D. Gonsalves, Cornell University, Geneva, USA) reacted strongly with BlCMV-5. No reaction to BlCMV-5 antigen was detected when the antiserum to the Morocco isolate of cowpea aphid-borne mosaic virus (CAMV) from D. Gonsalves was used.

BlCMV and CAMV are two potyviruses pathogenic to cowpea and asparagus bean. Lima *et al.* (1979) suggested that BlCMV and CAMV should be considered as distinct members of the potyvirus group on the basis of the serological tests. Results from our studies clearly indicated that the isolate from Thailand was BlCMV.

Serological comparison of SMV, BCMV and BlCMV

The serological relationships among SMV, BCMV and BlCMV were established using double immunodiffusion tests. The medium consisted of 0.8% agar, 0.85% sodium chloride, 0.5% lithium 3,5-diiodosalicylate and 0.1% sodium azide. The results of reciprocal comparison of the isolates indicated that BLCMV-5, BlCMV-J (an isolate of BlCMV from Japan), BCMV-12, BCMV-J and azuki bean mosaic virus (AzMV) were serologically identical (Table 3). Antisera to SMV-H and SMV-27 reacted weakly with BlCMV and BCMV, with homologous reaction lines spurring over heterologous lines. The results of reciprocal serological tests with other potyviruses indicated that BlCMV, BCMV and SMV were not serologically related to potato virus Y and turnip mosaic virus.

Hollings and Brunt (1981) suggested that many of potyviruses are serologically interrelated and, from the closeness of the relationships, some could well be considered as virus strains or serotypes rather than distinct viruses. BlCMV, BCMV and AzMV may be in future considered as virus strains rather than distinct viruses.

Table 3 Serological reaction among bean common mosaic virus (BCMV), blackeye cowpea mosaic virus (BlCMV), soybean mosaic virus (SMV) and some other potyviruses in agar gel diffusion plates containing 0.5% lithium 3,5-diiodosalicylate

Antisera	Virus isolates								
	BCMV-12	BCMV-J	BlCMV-5	BlCMV-J	SMV-27	SMV-H	AzMV	PVY	TuMV
BCMV-12	+	+	+	+	+*	+*	+	-	-
BCMV-J	+	+	+	+	+*	+*	+	-	-
BlCMV-5	+	+	+	+	+*	+*	+	-	-
BlCMV-J	+	+	+	+	+*	+*	+	-	-
SMV-27	+*	+*	+*	+*	+	+*	+*	-	-
SMV-H	+*	+*	+*	+*	+*	+*	+*	-	-
AzMV	+	+	+	+	+*	+*	+	-	-
PVY	-	-	-	-	-	-	-	+	-
TuMV	-	-	-	-	-	-	-	-	+

AzMV : azuki bean mosaic virus, PVY : potato virus Y, TuMV : turnip mosaic virus. * Spur reaction was observed with the virus isolate homologous to the antiserum used.

References

1) Deema, N. (1976): Virus diseases of economic crops in Thailand. Trop. Agr. Res., Series No. 10. 69–72.
2) Hollings, M. and Brunt, A. A. (1981): Potyviruses. Handbook of plant virus infections comparative diagnosis. North-Holland Biomedical Press, Amsterdam, The Netherlands. pp. 731–807.
3) Kaiser, W. J. and Mossahebi, G. H. (1974): Natural infection of mungbean by bean common mosaic virus. Phytopathology, 64, 1209–1214.
4) Lima, J. A. A., Purcifull, D. E. and Hiebert, E. (1979): Purification, partial characterization, and serology of blackeye cowpea mosaic virus. Phytopathology, 69, 1252–1258.

Discussion

Makkouk, K.M. (ICARDA): The serological data presented would tend to suggest that azuki bean mosaic virus, some strains of bean common mosaic virus and blackeye cowpea mosaic virus are not only closely related but can be regarded as one virus.