Blackgram Mottle Virus Occurring on Mungbean and Soybean in Thailand

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Introduction

In 1979, the authors collected blackgram and mungbean plants showing mottling and mosaic symptoms from the southern part of Northern Thailand. The preliminary studies carried out by the authors indicated that the causal virus had icosahedral morphology and induced symptoms in certain hosts unlike those of the known viruses infecting blackgram or mungbean.

After our preliminary studies, an isometric virus, blackgram mottle virus (BLMV), was reported from India.[1] BLMV is transmissible by the bean leaf beetle (*Cerotoma trifurcata* Forst.) and the Mexican bean beetle (*Epilachna varivestis* Muls.), and by mechanical inoculations in greenhouse.[2] The beetle transmissibility and serological properties indicated that BLMV is a member of the beetle-transmitted virus groups.

In the present report properties of the virus isolated from mungbean and soybean in Thailand indicating that it is beetle transmissible and serologically related to BLMV from India will be described.

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Materials and methods

1) Virus source and maintainance

The original virus source was from naturally infected mungbean plants collected from Northern Thailand. During our subsequent surveys, infected soybeans were also collected in farmer's fields in 1980. Both the mungbean and the soybean isolates of the virus (BLMV-M and BLMV-S, respectively) were each passed through five serial single-lesion transfers in *Phaseolus vulgaris* L. cv. 'Top Crop'. Virus isolates were maintained in *P. vulgaris* L. 'Yamashiro Kurosando' by successive mechanical inoculations, which was achieved by rubbing the carborundum (600 mesh)-dusted leaves with a piece of cotton dipped in the homogenate of infected leaves in 0.1 M sodium phosphate buffer, pH 7.2, containing 0.1% thioglycollic acid.

2) Host range and physical properties

Host range of both BLMV-M and BLMV-S was determined by mechanical inoculations to various plant species (Table 1) in the greenhouse. Virus physical properties (dilution end point, thermal inactivation point, and longevity *in vitro*) were determined by the conventional procedures using *P. vulgaris* L. 'Tsurunashi Kintoki' as test plants.

3) Insect transmission

Virus-free beetles, *Monolepta signata* Oliv., and aphids, *Aphis craccivora* Koch, were allowed an acquisition access of 1 day on infected mungbean plants in tubular plastic cages. Inoculation was done immediately after acquisition by allowing beetles or aphids an overnight access on healthy mungbean plants. Two or 10 to 20

beetles or aphids, respectively, were used per plant.

4) Virus purification and electron microscopy

Virus was purified by extracting sap from Yamashiro Kurosando leaves at 10–16 days after inoculation in 0.1 M sodium phosphate buffer, pH 7.2, containing 0.1% thioglycollic acid (1.0–1.5 m*l* buffer per g tissue). The sap was clarified by addition of 1 m*l* of chloroform-butanol mixture (1 : 1, v: v) per g tissue and centrifugation at 6,000 g for 10 min. The clarified sap was recovered from the aqueous phase and subjected to two cycles of differential centrifugation (ultracentrifugation and high-speed centrifugation at 100,000 g for 1 hr and 10,000 g for 10 min, respectively). The final ultracentrifugation pellets were resuspended in 0.1 M sodium phosphate buffer, pH 7.2, and analyzed by density gradient centrifugation (Hitachi RPS 27–2 rotor, 26,000 rpm for 6 hr) through 10–40% linear sucrose density gradients prepared in the same buffer. Fractions containing virus were collected, pooled and concentrated by centrifugation as above. Virus pellets were resuspended in 0.05 M sodium phosphate buffer, pH 7.2.

Samples for electron microscopy were mounted on carbon-stabilized, formvar coated grids and stained with neutral 2% phosphotungstic acid. Observations were done in a Hitachi Model H 500 electron microscope.

5) Serological tests

Serological relationship of BLMV-M and BLMV-S was determined by Ouchterlony double-diffusion tests[3] using antiserum against the Indian isolate of BLMV. Reactions of BLMV-S to antisera against other beetle- and whitefly-borne viruses, namely, southern bean mosaic virus, turnip yellow mosaic virus, radish enation mosaic virus, squash mosaic virus and cowpea mild mottle virus, were also tested.

Results and discussion

1) Host range and physical properties

Among 35 plant species in nine families tested, both BLMV-M and BLMV-S had similar host reactions. All leguminous plants except *P. lunatus* were infected with the virus (Table 1). Infected blackgram and mungbean showed chlorotic spots with some necrotic specks on the inoculated leaves. Systemic symptoms were mild mottling or mosaic. Tsurunashi Kintoki bean which was used as assay host showed pinpoint necrotic lesions on the inoculated leaves (Plate 1, A) without any systemic symptoms. Yamashiro Kurosando bean, the host used for virus propagation, showed local chrolotic spots (plate 1, B) 3–4 days after inoculation, with mosaic (Plate 1, C) or leaf roll (Plate 1, D) symptoms on the emerging trifoliolate leaves.

BLMV isolated from India did not infect Lee soybean,[2] however, we have isolated BLMV-S from naturally infected soybeans in Thailand. In our host range tests, both BLMV-M and BLMV-S induced local necrotic lesions on soybeans from Japan with no systemic symptoms and systemically infected SJ 1, SJ 2 and SJ 4 soybeans from Thailand. We therefore concluded that soybean reactions to BLMV infection depended upon the cultivars used.

BLMV-M and BLMV-S had a dilution end point of 10^{-9}–10^{-10}, a thermal inactivation point of 85–90°C for 10 min, and a longevity *in vitro* of 6–9 weeks at 20°C.

2) Insect transmission

Aphis craccivora did not transmit the virus, but *Monolepta signata* transmitted the virus at a rate of about 20% (eight out of 41 plants inoculated showed symptoms typical of BLMV). Plants exposed to beetles without acquistion feeding access remained healthy. From observations made during our surveys we noticed the prevalence of *M. signata* by the characteristic feeding injuries in both the mungbean or blackgram and soybean fields. Insecticides are not always used by farmers due to the low return of the crops. Therefore, the possibility of BLMV becoming a limiting factor in mungbean, blackgram and soybean production in Thailand is very likely because the virus is occurring quite frequently on mungbean and blackgram plants in major planting areas, and also the soybean cultivars used by farmers in Thailand are the SJ series which we found in our host range test susceptible to the virus.

Table 1. Host range of blackgram mottle virus isolated from Thailand

Tested plants		Symptoms[a]	
Family	Species	Inoculated leaves	Uninoculated leaves
Aizoaceae	*Tetragonia expansa*	Lc	—
Amaranthaceae	*Gomphrena globosa*	Ln	—
Chenopodiaceae	*Chenopodium amaranticolor*	Lc	—
	C. quinoa	Lc	—
	Spinacia oleracea	—	—
Compositae	*Zinnia elegans*	—	—
Cruciferae	*Brassica rapa*	—	—
Cucurbitaceae	*Cucumis sativus*	—	—
Leguminosae	*Arachis hypogaea*	Ln	—
	Cajanus cajan	l	—
	Cassia tora	l	—
	Dolichos lablab	Ln	—
	Glycine max 'Okuhara Wase'	Ln	—
	'Toyosuzu'	Ln	—
	'SJ 1, SJ 2, SJ 4'	l	mo
	Phaseolus anguralis	l	VC
	P. lunatus	—	—
	P. vulgaris 'Top Crop'	Ln	—
	'Tsurunashi Kintoki'	Ln	—
	'Master Piece'	Lc	Mo, LR
	'Yamashiro Kurosando'	Lc	Mo, LR
	'Wakaba'	Lc	Mo, LR
	Pisum sativum	l	—
	Vicia faba	l	—
	Vigna mungo	Ln	Mo
	V. radiata 'M7A'	Ln	Mo
	V. sesquipedalis 'Kurodane Sanjaku'	Ln	—
	V. sinensis 'Black Eye'	l	—
Pedaliaceae	*Sesamum indicum*	l	s
Solanaceae	*Datura stramonium*	—	—
	Lycopersicon esculentum	—	—
	Nicotiana clevelandii	l	—
	N. glutinosa	—	—
	N. tabacum 'Bright Yellow'	—	—
	Petunia hybrida	—	—

a) Key to symptoms: l=symptomless local infection, Lc=chlorotic local lesions, Ln=necrotic local lesions, LR=leaf roll (downward), Mo=mottle, mo=mild mottle, s=symptomless systemic infection, VC=vein clearing, —=no infection.

3) Virus purification and electron microscopy

Both BLMV-M and BLMV-S exhibited single ultraviolet light (254 nm) absorbing zone in sucrose density gradient profiles. The virus had an ultraviolet light absorption spectrum typical of nucleoprotein components with a A_{260}/A_{280} ratio of about 1.55. Using the extinction coefficient ($E^{0.1\%}_{260\,nm}$) value of 5.0 reported for BLMV from India,[2] the yields of both BLMV-M and BLMV-S were in the range of 50–90 mg/kg tissue.

Under the electron microscope, purified virus preparation showed isometric particles with a diameter of about 28 nm (Plate 2). Some of the particles were penetrated by stain suggesting that they were empty capsids but our repeated attempts to detect different sedimentation components of the virus either by zone density

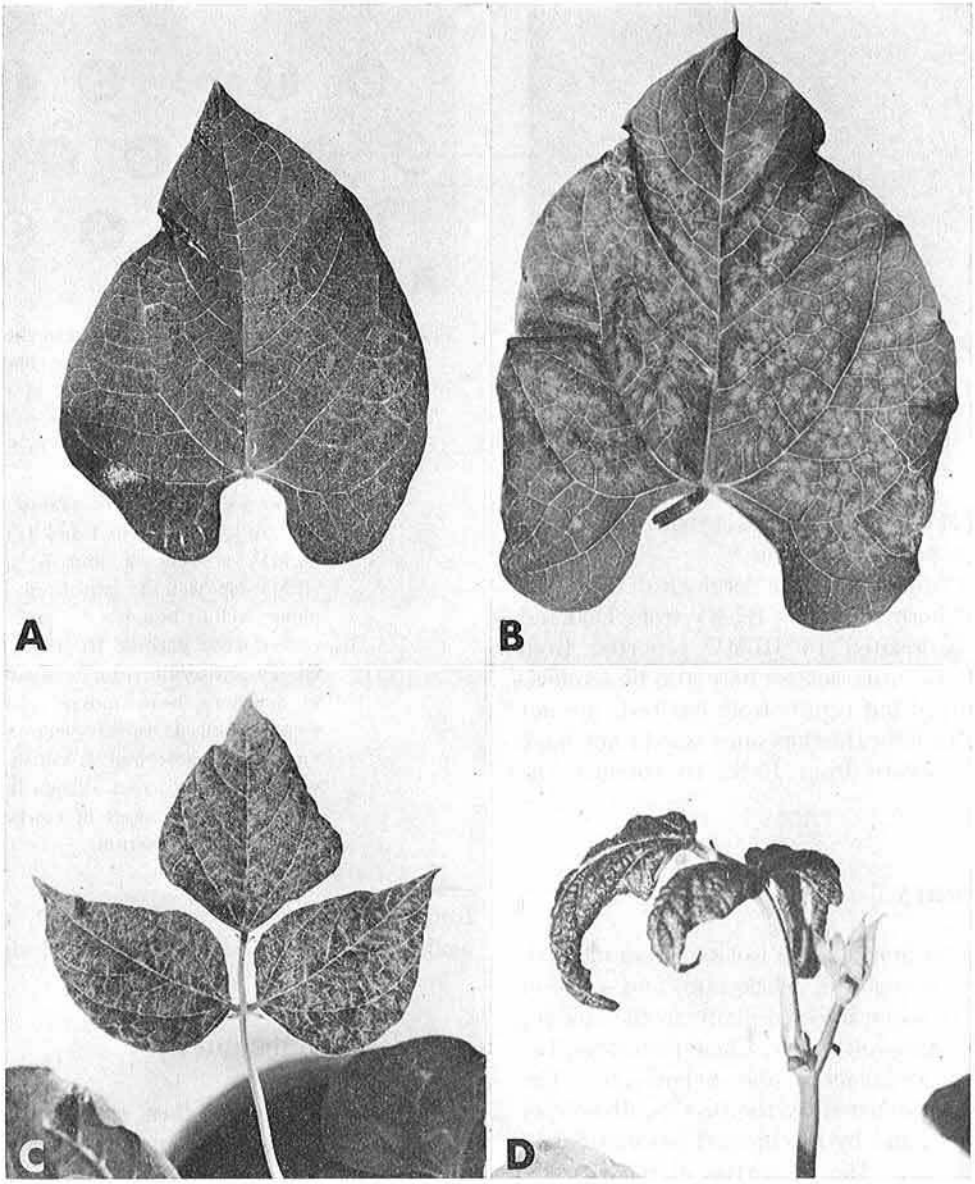

Plate 1. Symptoms in *Phaseolus vulgaris* caused by infection of blackgram mottle virus isolatted from Thailand. A, pinpoint necrotic lesions on the inoculated leaf of Tsurunashi Kintoki bean; B, C and D, local chlorotic lesions, mosaic, and leaf roll, respectively, in Yamashiro Kurosando bean.

gradient or equilibrium centrifugation failed. BLMV reported from India also has a single component when subjected to analytical ultracentrifugation.[2)]

3) Serology

In Ouchterlony double-diffusion tests using antiserum against BLMV from India, BLMV-M and BLMV-S showed reaction of identity (Plate 3, A). BLMV-S reacted only with antiserum against BLMV from India, but did not react with antisera against southern bean mosaic, turnip yellow mosaic, radish mosaic, squash mosaic, or cowpea mild mottle virus (Plate 3, B). In other

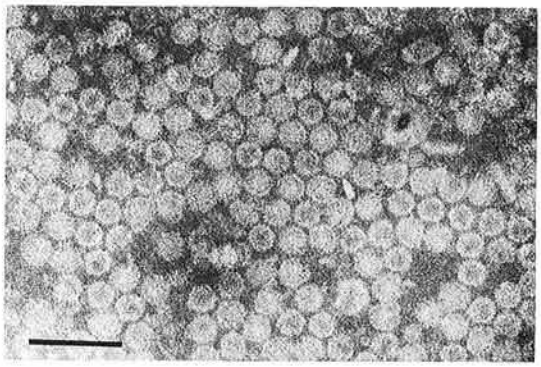

Plate 2. Electron micrograph of partially purified blackgram mottle virus particles negatively stained with 2% sodium phosphotungstate, pH 6.9. Bar equals 100 nm.

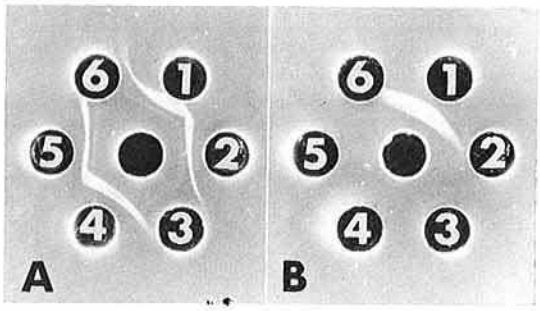

Plate 3. Serological relationship between the mungbean and soybean isolates of blackgram mottle virus and the reaction of the soybean isolate to antisera against some beetle- and whitefly-borne viruses in Ouchterlony double-diffusion tests.

A, center well: antiserum against BLMV (Indian isolate); wells 1 and 4: purified BLMV-S; wells 2 and 5: purified BLMV-M; well 3: sap from healthy plant; well 6: buffer.

B, center well: purified BLMV-S; well 1: BLMV-antiserum (Indian isolate); well 2: southern bean mosaic virus-antiserum; well 3: turnip yellow mosaic virus-antiserum; well 4: radish mosaic virus-antiserum; well 5: squash mosaic virus-antiserum; well 6: cowpea mild mottle virus-antiserum.

tests, BLMV-M did not react with antiserum against cowpea mosaic virus.[4)]

Results from the above serological test indicated that both isolates of BLMV from Thailand are closely related to BLMV reported from India. Both virus isolates may also be serologically identical but results from our tests are not available to indicate this since we do not have the virus isolate from India to conduct the reciprocal tests.

Summary

Blackgram mottle virus isolated from naturally infected mungbean, blackgram, and soybean plants in Thailand infected plants in the families Aizoaceae, Amaranthaceae, Chenopodiaceae, Leguminosae, Pedaliaceae and Solanaceae. The virus was transmitted by the beetles, *Monolepta signata* Oliv., and by mechanical inoculations in the laboratory. The properties of the virus in plant sap are a dilution end point of 10^{-9}–10^{-10}, a thermal inactivation point of 85–90°C for 10 min, and a longevity *in vitro* of 6–9 weeks at 20°C. Purified virus preparations had an ultraviolet light absorption spectrum typical of nucleoprotein components with a A_{260}/A_{280} value of about 1.55. Purified virus preparations contained isometric particles with a diameter of about 28 nm. BLMV isolated from mungbean and soybean in Thailand showed reaction of identity in Ouchterlony double-diffusion tests using antiserum against the BLMV previously reported from India, but did not react with antisera against some other beetle-transmitted viruses.

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