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Blackeye Cowpea Mosaic Virus (BlCMV) in Yard-long Bean in the Mariana Islands

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Abstract—A mosaic disease of *Vigna unguiculata* subsp. *sesquipedalis* (asparagus bean or yard-long bean) was determined via host range studies, ring interface and PAS-ELISA tests to be consistent with the blackeye cowpea mosaic virus (BlCMV). Sap inoculations of 39 legumes resulted in 31 becoming infected, as did 9 of 24 non-legume species. Seed transmission occurred in 37% of yard-long bean seeds tested. The virus was transmitted by apterous *Aphis craccivora* in a non-persistent fashion. Seed and aphid transmission are consistent with BlCMV. Other important crop plants infected included snap beans, winged bean, and yam beans (jicama). This is the first report of BlCMV on winged bean (*Psophocarpus tetragonolobus.* Other less important crop plants infected were mung bean and sunnhemp. Of eleven yard-long bean cultivars tested with sap-inoculation, none showed resistance. Cultivar Green Arrow (Known-You Seed Co.) performed well with low disease incidence and high yields in field tests over 2 seasons.

Introduction

Yard-long and asparagus bean are two of the many common names given to *Vigna unguiculata* (L.) Walp. subsp. *sesquipedalis* (L.) Verdc. The species is well adapted in the Mariana Islands and is widely grown for local human consumption. Although common snap bean (*Phaseolus vulgaris* L.) is also grown, yard-long bean is more popular because it grows better at lower elevations.

Reports of a mosaic disease of bean on Guam date back to 1921 (Anonymous 1922). Today crops of yard-long bean have a high incidence of mosaic (Kimmons et al. 1990, Wall & Kimmons 1991). In a 1988 survey, mosaic and powdery mildew were the most prevalent disease problems of yard-long bean on Guam. In early tests in 1988 the causal agent of mosaic was transmitted mechanically and by aphids; therefore a virus was suspected. Identification was essential for its control. The purpose of this study was to identify the causal agent of the mosaic disease, and to screen yard-long bean varieties for resistance to it.

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Materials and Methods

MECHANICAL TRANSMISSION

Leaves of yard-long bean plants exhibiting mosaic symptoms were collected from the farm of A. Manibusan in Mangilao, Guam, and used to inoculate 7-day-old seedlings of *V. unguiculata* subsp. *sesquipedalis* cv Local Red. Infected leaves were triturated in mortar and pestle with phosphate buffer, pH 7.9. Leaves of test seedlings were dusted with carborundum and mechanically inoculated. Plants were then grown in aphid-proof cages in a greenhouse along with control plants, which had not been inoculated.

VIRAL ISOLATION

Sap from inoculated symptomatic plants was used to inoculate *Chenopodium amaranticolor* Coste & Reyn., a local lesion host. Three successive single-lesion transfers were made on *C. amaranticolor,* and a single lesion was used to inoculate 7-day-old seedlings of yard-long bean cv Local Red. The single-lesion isolate was propagated and maintained in yard-long bean and in *Nicotiana benthamiana* Domin.

HOST RANGE

Three to eight test plants each of 63 accessions comprising several species and varieties were sap-inoculated as above, using young systemically infected leaves of yard-long bean. Plants were maintained in a screenhouse. All tests were repeated at least twice. After 24–30 days, test plants were indexed for infection by back-inoculation to *C. amaranticolor* or by PAS-ELISA.

APHID TRANSMISSION

Apterous *Aphis craccivora* Koch from virus-free cultures were starved for 1 hr, then given 30 sec to 2 min acquisition access on symptomatic yard-long bean. Aphids were then transferred to 8 healthy 7-day-old yard-long bean seedlings, five aphids per plant, for inoculation-feeding periods of 4–6 hrs. Control plants received aphids from asymptomatic plants.

SEROLOGY

Assays of raw sap were carried out by Protein A sandwich ELISA (PAS-ELISA). This procedure, also known as $F(ab')_2$ indirect ELISA (Edwards & Cooper 1985, Reddick et al. 1991) was modified. The procedure was as follows.

Polystyrene microtiter plates (Dinatech, McLean, VA) were first coated with protein A (Sigma Chemical Co.) diluted 1:1000 in carbonate buffer (0.05*M* Na_2CO_3 plus 0.02% $NaNO_3$, pH 9.6) for 2 hr at 28 C. Plates were rinsed 4 times (all subsequent rinses were done in the same manner), with phosphate buffered saline plus Tween 20 (PBST). Antiserum was added in a 1:1000 dilution with PBST. Plates were incubated again for 2 hr at 28 C, then rinsed again. Test samples extracted in 1:5 PBST were then added and incubated overnight at 4 C. In the morning they were again rinsed. Antiserum was again added as before, and

incubated again for 2 hr at 28 C. Plates were then rinsed. Alkaline phosphatase-conjugated protein A (Sigma Chemical Co.) was added (0.1 mg in 250 microliters PBST, then diluted 1:1000) and incubated for 2 hr at 28 C. Plates were again rinsed. Substrate (Sigma 104 phosphatase substrate tablets) was added (1 mg per ml of 10% Diethanolamine adjusted to pH 9.8) and kept at room temperature for 1–2 hr. Reaction was stopped with 50 microliters of 3*M* NaOH per well. Reactions were read visually and with an ELISA reader (Uniskan I, Labsystems, Finland) at A_{405}.

Antigen and antisera for the NLS strain of bean common mosaic virus (BCMV) and strain W of BlCMV were obtained from L. Bos, Research Institute for Plant Protection, Wageningen, The Netherlands. Antisera for BlCMV, cowpea severe mosaic virus (CSMV), southern bean mosaic virus (SBMV), cowpea chlorotic mottle virus (CCMV), tobacco mosaic virus (TMV) legume strain, and pea mosaic virus (PMV) strain n were obtained from C. W. Kuhn, University of Georgia, Department of Plant Pathology, Athens. The antigens and antisera obtained from Bos, plus that of BlCMV from Kuhn were used in PAS-ELISA tests. The rest were used in ring interface tests.

Ring interface tests were carried out according to the procedure described by Hampton et al. (1990). Filtered and centrifuged extracts of plant samples were layered on top of a 1:10 dilution of antisera in phosphate buffered saline plus 10% glycerin in small glass tubes, 0.5 × 5 cm.

SEED TRANSMISSION

Seed of commercially available yard-long bean variety Greenpod Kaohsiung (Known-You Seed Co., Taiwan) were purchased from a local source. A total of 300 seeds in various tests were planted in flats and kept in aphid-proof cages. Seed from mechanically inoculated and control plants of yard-long bean grown in the screenhouse were also collected. Equal numbers of seeds from inoculated and control plants were grown in the screenhouse and tested for virus infection at the first true leaf stage by PAS-ELISA. The test was repeated with seed from field-infected plants.

SCREENING FOR RESISTANCE

Eleven varieties of yard-long bean were tested in the screenhouse for resistance to the mosaic disease. Varieties tested were: Ferry Morse Asparagus bean (FA), Surinam (SU), Burpee Asparagus bean (BA), Local Bush or Dwarf bean (BU), Local Red, collected from J. Meno, Malojloj, Guam (LR), Local Black, from J. Cruz, Malesso, Guam (LB), Known-You Green Arrow (KYA), Known-You Greenpod Kaohsiung (KYK), Local Green, from A. Manibusan, Mangilao, Guam (MG), Local White, A. Manibusan (MW), and Extra Long Red Seed from Takii & Co., Japan (TR). Seedlings were grown in 5 cm diameter pots, in sterile potting mix, with 12 plants/plot and 2 replicates per variety. Plants were sap-inoculated; initially a 1:5 sap to buffer ratio was used. In a second trial, the ratio was changed to 1:10.

Field tests for resistance to the mosaic disease were carried out at the Manibusan farm under natural disease pressure. All varieties from the screenhouse tests, except for Ferry Morse Asparagus and Surinam, were included. Plots consisted of single rows of hills. Rows were 5 m long and spaced 1 m apart. Hills within rows were 20 cm apart. Plantings were thinned to 2 plants per hill. Three replications were used in a completely randomized design. Fertilizer (16N:16P:16K) was applied in a side-dressed band (local practice) at the recommended rates at 2 and 6 weeks after planting. Irrigation was by dripline. All agronomic practices were done as is customary for local growers. The field was planted December 1, 1989 (end of wet season). The experiment ended on March 6, 1990. The number of plants with mosaic symptoms was recorded for each plot. A second experiment with the same varieties was carried out in an adjacent field. It began with planting on April 4, 1990, and ended on July 16, 1990. Distance between rows was changed to 1.5 m.

Statistical analyses were made with software from either NCSS (J. I. Hintze, Kaysville, UT) or SuperAnova (Abacus, Berkeley, CA). Percent incidence data were subjected to square root transformations before performing analysis of variance (Gomez & Gomez 1984).

Results

MECHANICAL TRANSMISSION

Yard-long bean plants inoculated with sap from diseased plants and grown in the screenhouse developed systemic symptoms resembling those observed in growers' fields. Symptoms developed in 75% of the inoculated plants and included mosaic, dark green vein-banding, rugosity and epinasty.

HOST RANGE

Thirty-one of 39 possible legume hosts developed symptoms or were asymptomatically infected, as verified by back-inoculation to *C. amaranticolor*. All cultivars of yard-long bean developed systemic symptoms. Nine of 24 non-legumes were infected (Table 1). Symptomless infections were detected in New Zealand spinach (*Tetragonia tetragonioides* (Pall.) Kuntze), fenugreek (*Trigonella foenum-graecum* L), and Mississippi Silver cowpea (*V. unguiculata* (L.) Walp.).

APHID TRANSMISSION

Five of 8 plants (63%) receiving aphids from symptomatic plants developed characteristic mosaic symptoms after 7–14 days at all tested acquisition times. Control plants did not develop symptoms.

SEROLOGY

In PAS-ELISA, antisera for both strains of BlCMV gave positive reactions with sap from mechanically inoculated yard-long bean plants, and with the positive controls. No reaction to healthy yard-long bean plants occurred. Sap from inoculated *N. benthamiana* plants also reacted positively to these antisera. No

Table 1. Reaction of 63 plant species, cultivars, or varieties mechanically inoculated with BlCMV.

Family Species Cultivar	Reactions	
	Local	Systemic
Aizoaceae		
Tetragonia tetragonioides		
New Zealand spinach	+	+
Amaranthaceae		
Amaranthus spinosus	–	–
Beta vulgaris		
Detroit Dark Red	–	–
Gomphrena globosa		
Mixed	–	Mo
Plant Virus	+	–
Spinacea oleracea		
Bloomsdale Long Standing	±	±
Araliaceae		
Polyscias sp.	–	–
Asteraceae		
Synedrella nodiflora	*	±
Bignoniaceae		
Tecoma stans	–	–
Chenopodiaceae		
Chenopodium amaranticolor	YL	–
Chenopodium quinoa	YL	–
Cucurbitaceae		
Cucumis sativus		
Hybrid Sweet Salad	–	–
Chicago Pickling	–	–
Cucubita pepo		
Ambassador	–	–
Luffa acutangula		
Luffa gourd	–	–
Fabaceae		
Arachis hypogea		
Spanish Peanut	–	–
Starr	–	–
Cassia alata	–	–
Crotalaria juncea		
Tropic Sun sunnhemp	NL	Mo, LR, LC
Glycine max		
Bragg	+	–
Vinton	±	–
Medicago sativa		
Du Puis	–	–
Pachyrhizus erosus	*	±
Phaseolus lunatus		
Henderson Bush Lima	RV, NS, N	M, C, Dw, N

Table 1. Continued.

Family Species Cultivar	Reactions Local	 Systemic
P. vulgaris		
Black Turtle	Mo	Mo, Dw, N
Greencrop	+	Mo, Dw, N
Pisum sativum		
Perfected Wales	–	±
Wando	*	–
Psophocarpus tetragonolobus	*	M
Trigonella foenum-graecum	+	+
Vicia faba		
Fava Bush Long Pod	RL	+
V. faba minor		
Bell bean	RL	–
Vigna. radiata		
Berken mung bean	–	Mo
V. unguiculata		
California Blackeye #5	+	M
Mississippi Silver	±	+
Purple Hull	–	–
Queen Ann	±	±
Serido	*	–
Sete semanas	*	–(1/6 VB)
V. unguiculata subsp. *sesquipedalis*		
Burpee Asparagus bean	*	M, VB, Ru
Dow Gauk	*	M, VB, Ru
Extra Long Red Seed	*	M, VB, Ru
Green Arrow	*	M, VB, Ru
Green Pod Kaohsiung	*	M, VB, Ru
local black	*	M, VB, Ru
local bush	*	M, VB, Ru
local green	*	M, VB, Ru
local red	*	M, VB, Ru
local white	*	M, VB, Ru
Maagap	*	M
Mabun JI	*	M, YS
Sandigan Pole	*	M
Sumilang	*	M, Mo
Surinam	*	M
Lamiaceae		
Ocimum basilicum		
Sweet basil	*	–

Table 1. Continued.

Family Species Cultivar	Reactions Local	 Systemic
Poacea		
Triticum aestivum		
Minter Winter wheat	–	–
Solanaceae		
Datura stramonium		
Plant Virus	–	–
Lycopersicon esculentum		
Marglobe	–	–
Nicotiana benthamiana	*	M, VB, LC
N. glutinosa	–	–
N. tabacum		
Hicks Broadleaf	*	–
Samsun	–	–
Petunia hybrida		
Plum Pudding	+	–

Legend:

C—chlorosis of leaves
D—leaf deformity
Dw—Dwarfing of leaves
LC—leaves curling downward
LR—leaf rolling upward
M—Mosaic
Mo—mottle
N—necrosis of plant
NL—necrotic local lesions
NS—necrotic spots
RL—red local lesions
Ru—rugosity
RV—red vein banding
VB—dark green vein banding
YL—yellow local lesions
YS—yellow spots

\+ symptomless infection (positive in back inoculation or ELISA)
– no symptoms (negative in back inoculation or ELISA)
± positive, but not in all back inoculations or ELISA
* no symptoms, no back inoculation or ELISA.

positive reactions were observed with the other antisera, except for positive controls. In ring interface tests, yard-long bean samples gave positive results with antiserum for BlCMV only. Uninoculated controls gave negative results.

SEED TRANSMISSION

Tests on plants from nursery-purchased seed were negative except for 1 questionable infection out of 300 plants. Fifteen of 41 plants (37%) grown from seed of infected screenhouse-grown plants tested positive for BlCMV in PAS-ELISA and showed mosaic symptoms on the first true leaves. Control plants showed no symptoms and tested negative. Sixteen of 50 (32%) seedlings from field-infected seed showed symptoms.

SCREENING FOR RESISTANCE

No significant differences in disease incidence were found among varieties in the first screenhouse test. All entries were infected. The weaker dilution used for

inoculating the second test resulted in milder symptoms; however, no differences in disease incidence were found.

In the first field test, a positive correlation ($r = 0.780$) was found between visual scores of mosaic incidence and yield. Varieties with significantly higher visual mosaic scores yielded significantly higher. In the second experiment, there was also a positive correlation between visual scores of mosaic incidence and yield ($r = 0.538$), but there was a negative correlation between infection scores from PAS-ELISA and yield ($r = -0.393$); although the correlation was low, it was significant at the .05 level. Correlation of visual scores with PAS-ELISA data was also low ($r = 0.385$), yet significant. The highest yields were obtained by MW, LR, KYA and KYK in the first test, and MG, BA, TR, and KYA in the second (Tables 2, 3).

On California Blackeye Cowpea #5, our test isolate produced mosaic but no downward cupping of leaves on all test plants, nor did this occur on *N. benthamiana,* although this species did develop green vein-banding. On Serido cowpea, no infection developed, while on Sete Semanas cowpea, only 1 of 6 test plants developed vein banding.

Discussion

Results from all our tests agree with previous descriptions of BlCMV, indicating that the causal agent of the mosaic disease affecting yard-long bean is a strain of this potyvirus (Purciful & Gonsalves 1985, Taiwo et al. 1982, Dijkstra et al. 1987). It is important to note, however, that Dijkstra et al. (1987) suggest that BlCMV and cowpea aphidborne mosaic virus (CAMV) should be considered synonymous until further work can elucidate their separate identities. If so, the name BlCMV then takes precedence. While Taiwo et al. (1982) considered that they found some cowpea cultivars capable of differentiating between the two vi-

Table 2. Yields of 9 yard-long bean cultivars at Mangilao, Guam, planted in Dec. 1989 (Test 1) and April 1990 (Test 2).

Cultivar	Yield T/ha		Mean
	Test 1*	Test 2	
MW	18.46a	2.96b	10.71a
KYA	15.96ab	4.70ab	10.33a
LR	17.13a	3.43b	10.88a
MG	11.19bc	8.20a	9.70a
KYK	12.51abc	3.86b	8.19a
TR	10.95bc	4.87ab	7.91ab
LB	11.44bc	3.12b	7.28ab
BA	8.80cd	5.22ab	7.01ab
BU	3.66d	3.77b	3.72b

* Means followed by different letters are different at .05 level (Fisher's Protected LSD).

Table 3. Incidence of BlCMV in field trials of 9 yard-long bean cultivars at Mangilao, Guam, planted in Dec. 1989 (Test 1) and April 1990 (Test 2)

	% Incidence-Visual Score				% Incidence
Cultivar	Test 1	Test 2	Mean*	Cultivar	ELISA Test 2
BU	1.1	5.3	3.2a	BA	26a
TR	5.6	1.3	3.4a	TR	39a
BA	6.7	0.6	3.6a	KYA	46a
LB	7.8	0.3	4.0a	LR	56a
KYK	10.0	3.6	6.8ab	MG	57a
KYA	14.4	0	7.2ab	LB	65ab
LR	15.6	4.6	10.1bc	MW	73ab
MW	21.1	2.6	11.9bc	BU	88b
MG	15.6	9.3	12.4c	KYK	88b

Legend:
*Fisher's Protected LSD, at .05 significance level.
KYA: Known-You Green Arrow
KYK: Known-You Greenpod Kaohsiung
TR: Takii Extra Long Red
BA: Burpee Asparagus
LR: Local Red
LB: Local Black
MG: Manibusan Green
MW: Manibusan White
BU: Bush

ruses in question, Dijskstra et al. point out that this work was based on very few plants being tested, in some cases as few as 3. Furthermore, they argue, when they tried to duplicate these tests using 30–50 plants per cultivar, they got varied results. Because current information in the literature can be confusing in this respect, we chose to follow the suggestion of Dijkstra et al. and call our isolate BlCMV.

The fact that BlCMV is seed-transmitted is of particular importance. Local growers traditionally save their own seed because their favorite varieties are not commercially available. Incidence rates of mosaic nearing 100% are not unusual on Guam. Such a high incidence can easily be explained if seed transmission is occurring. None of the other plants in the host range study are ubiquitous enough to be important weed hosts for this virus in our region. Other crop plants of some importance (snap, yam and winged beans) were found to be hosts for BlCMV, and could act as sources of inoculum for yard-long bean. Yam and winged beans can grow year-round on Guam, and they may therefore have a role as interseasonal sources of inoculum. However, with seed and aphid transmission, yard-long bean is very likely its own primary source of inoculum. If this is the case, then effective control of BlCMV on yard-long bean is dependent on a combination of using clean seed, early rogueing of any seed-infected seedlings, and aphid control, if possible.

Additional control steps that can be important are the selection of planting site, and prompt elimination of crop residues. In our region, because of limited land resources, growers may use the same or adjacent fields repeatedly. Planting away from older fields, which are likely to have infected plants, growers are less likely to get aphid-transmitted infections throughout the growing seasons. De-

struction of older fields as soon as possible would also be advisable to reduce the number of infected plants acting as sources of inoculum.

In the two field tests for resistance to BlCMV, visual scoring of incidence and yield data were not consistent. Regarding yield data, this was partly due to the differences in planting seasons. However, there were differences in disease incidence data that were simply due to the unreliability of visual scoring. Comparing both sets of incidence data for the second season, it is evident that visual scoring failed to detect all virus-infected plants. ELISA assays of virus diseases are more reliable than those based on visual symptoms because the former detect the presence of virus in plant samples, even if symptoms are not visible. Symptom expression of plant virus diseases is known to be affected by environmental conditions (Gibbs & Harrison 1976). Unfortunately, ELISA testing was not possible for the first field experiment.

Based on the available PAS-ELISA results, BA, TR, KYA, LR, and MG had low infection rates (Table 3). KYA, LR and MG had low incidence and produced good yields in both seasons (Table 2), making them the best overall cultivars tested. BA, although having the lowest incidence levels, has a purple tip and a shorter than standard pod length, which is undesirable for local markets, but would be a good choice for home gardens. MG and TR performed well under the drier climatic conditions of Test 2. MW and LR, on the other hand, did not. These cultivars might be improved through a breeding program in the future. Such a program could also incorporate BlCMV resistance available in cowpea germplasm, such as that seen in cultivars Serido and Sete Semanas. There are reports of at least 2 more resistant cowpea varieties (Kuhn et al. 1984).

Visual mosaic symptoms were positively correlated with yield, in spite of the expected negative correlation between yield and PAS-ELISA scores. A grower trying to estimate the extent of damage caused by mosaic and measuring disease by visual symptoms would be misled to believe there is no yield loss caused by this disease. Our PAS-ELISA data being negatively correlated with yield could be explained if BlCMV reduces yield. Subsequently, a separate test was performed to study the effect of mosaic infection on production (Wall & Kimmons 1991), which was found to be reduced considerably.

The PAS-ELISA technique developed in the course of this study has become a valuable research tool for working with this disease in our region. The choice of PAS-ELISA over double antibody sandwich (DAS) ELISA was made because it eliminates the need for conjugated antibody. Use of BlCMV antisera and PAS-ELISA now provides the ability to rapidly, accurately and cheaply perform virus-screening of yard-long beans from Guam, the Commonwealth of the Northern Mariana Islands and other island nations in the West and South Pacific regions. For instance, we have found BlCMV in yard-long bean samples from Saipan, where yard-long bean is also an important vegetable crop.

This is the first report of winged bean, *Psophocarpus tetragonolobus,* as a host of BlCMV. Further testing is needed to clarify the status of yam bean or jicama (*Pachyrhizus erosus*), *Synedrella nodiflora* and *Polyscias sp.* as hosts of this virus.

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