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Identification of blackeye cowpea mosaic virus from germplasm of yard-long bean and from soybean, and the relationships between blackeye cowpea mosaic virus and cowpea aphid-borne mosaic virus

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Abstract

Two potyvirus isolates, one from germplasm of yard-long bean (*Vigna unguiculata* ssp. *sesquipedalis*) introduced into the Netherlands, and another one from soybean plants (*Glycine max*) in Indonesia, were compared with two virus isolates of blackeye cowpea mosaic virus (BlCMV) from the USA and a Moroccan isolate of cowpea aphid-borne mosaic virus (CAMV). It is proposed that all five isolates be now considered BlCMV on the basis of host ranges, symptoms and serology. From our results, and a reassessment of the literature it is suggested to drop the name CAMV in favour of BlCMV.

Additional keywords: bean common mosaic virus, soybean mosaic virus.

Introduction

Yard-long bean or asparagus bean (*Vigna unguiculata* ssp. *sesquipedalis*) is a vegetable crop new to the Netherlands. Since 1981 it has been grown on a small scale in glasshouses for the production of green pods. In greenhouse varietal tests at the Institute for Horticultural Plant Breeding (IVT) at Wageningen seedlings of a number of germplasm entries showed symptoms of virus infection. At IPO such diseased plants were demonstrated to contain a potyvirus, possibly blackeye cowpea mosaic virus (BlCMV). At the same time in the Department of Virology of the Agricultural University a virus with similar morphology and host plant reactions was isolated from mosaic-diseased plants of soybean (*Glycine max*) from Indonesia. In its host reactions it differed from soybean mosaic virus (SMV) as described in the literature. The properties of both virus isolates were determined and compared with those of alleged isolates of BlCMV and the closely related cowpea aphid-borne mosaic virus (CAMV), viruses of which the original type strains are no more available. The results prompted reassessment of the literature on the distinction between BlCMV and CAMV.

Materials and methods

Source and maintenance of the virus isolates. The potyvirus isolates form yard-long bean seedlings at Wageningen and from soybean in Indonesia were designated W and Ind, respectively. One isolate of BlCMV from USA, designated Fla, was obtained from a few diseased plants of cowpea (*Vigna unguiculata* ssp. *unguiculata* cv. Knuckle Purple Hull) raised from seeds sent by Dr F.W. Zettler, Plant Pathology Department, University of Florida, Gainesville, USA, in response to our request for a representative inoculum of the Fla-isolate earlier described by Lima et al. (1979). A South Carolina isolate of BlCMV from cowpea, designated NR because of its causing necrotic ringspots on the leaves of several cowpea cultivars (Murphy et al., 1987), and a subculture of a Moroccan isolate of CAMV (Fischer and Lockhart, 1976), designated Mor, from cowpea, were obtained from Dr O.W. Barnett, Clemson University, Clemson, South Carolina, USA.

The five isolates were maintained in plants of *Nicotiana benthamiana* and cowpea cv. California Blackeye and in leaf material dried and stored over $CaCl_2$ at 4 °C. Research on these virus isolates was performed under licence of the Plant Protection Service of the Netherlands.

Inoculation. Manual inoculations were done using either purified virus or water-diluted crude sap from virus-infected plants, usually *N. benthamiana*, using carborundum (600 mesh) as the abrasive.

Plant material. In host-range tests three plants of each of 54 species or cultivars were inoculated. Of ten cowpea breeding lines from the germplasm collection of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, provided by Dr S. R. Singh, 30 to 50 plants per line were tested. All five virus isolates were inoculated to each species or cultivar on the same day. These tests were repeated twice at different times of the year.

Water-inoculated plants of the same species, cultivars and breeding lines served as controls. After about four weeks, return inoculations on *Chenopodium amaranticolor* and *C. quinoa* were done from both inoculated and non-inoculated leaves of symptomless plants, and also from the symptomless leaves of plants showing local infection only.

Properties in crude sap. Sap from infected leaves of *N. benthamiana* was used as inoculum and assayed on plants of *C. amaranticolor* and *C. quinoa*.

Transmission by aphids. Transmission experiments with *Myzus persicae* were performed with apterous aphids from virus-free cultures. Aphids were starved for 60 min and then given an acquisition access on young, diseased plants of *N. benthamiana* or cowpea for about 10 min. Then, these aphids were transferred to young, virus-free plants of *N. benthamiana* and cowpea, respectively, for an inoculation feeding period of about 20 min (6 or 12 plants with 10 aphids per plant). In order to exclude possible mechanical transmission of virus with the paint-brush used for aphid transfer, small pieces of adhesive tape were stuck onto the leaves of test plants and the aphids were placed on them. Finally, the aphids were killed with an insecticide (pirimicarb).

Purification. The five virus isolates were purified by the procedure of Lima et al. (1979) with some modifications. Leaves of *N. benthamiana* or cowpea were harvested about two weeks after inoculation and homogenized in a Waring Blendor with twice the amount (w/v) of 0.5 M potassium phosphate buffer, pH 7.5, containing 0.015 M sodium diethyldithiocarbamate (DIECA) and 1% (w/v) Na_2SO_3. The homogenate was squeezed through a double layer of cheesecloth and the resulting filtrate centrifuged at 9000 *g* for 10 min. n-Butanol was added to the supernatant at a final concentration of 7% (v/v). The mixture was stirred overnight at 4 °C and then centrifuged at 10 000 *g* for 15 min. The supernatant was filtered through cottonwool or glasswool, and the virus was precipitated by adding polyethylene glycol 6000 (PEG) to 7% (w/v). After stirring for about 90 min at 4 °C the precipitate was collected by centrifuging at 16 000 *g* for 15 min. The resulting pellet was resuspended in 0.02 M potassium phosphate buffer, pH 8.2, containing 0.1% (v/v) 2-mercaptoethanol and the suspension was stirred for three to four hours. For equilibrium density gradient centrifugation the suspension was made to 38% (w/v) CsCl and centrifuged in a Beckman SW 55 rotor at 147 000 *g* for 16 h. The resulting virus zone was removed from the centrifuge tube and dialysed against a series of changes of 0.02 M potassium phosphate buffer, pH 7.5. The purified virus suspensions were kept in liquid nitrogen to maintain their infectivity.

Ultraviolet-light absorption of purified virus suspensions was determined with a Gilford 2400-2 self-recording spectrophotometer. Absorbance values were not corrected for light scattering. Virus concentrations were determined using an extinction coefficient of 2.4 (mg $ml^{-1}cm^{-1}$) at 260 nm, the value determined for tobacco etch virus (Purcifull, 1966).

Molecular mass of the capsid proteins of the five virus isolates was determined by polyacrylamide gel electrophoresis on 10% (w/v) slab gels, as described earlier (Dijkstra et al., 1985).

Electron microscopy. Preparations of chopped leaves of infected plants and purified virus suspensions were made as described by Dijkstra et al. (1985). For *in situ* studies of pinwheel inclusions, diseased leaves of *N. benthamiana* were prepared as described by Jayasinghe and Dijkstra (1979).

Serology. For production of antisera to Fla, Mor and Ind, a mixture of 1 ml of a purified virus suspension (0.3 mg ml^{-1}) and 1 ml of Freund's complete adjuvant was subcutaneously injected into a rabbit. After 14 days, a second injection was given intramuscularly with 0.8 mg ml^{-1} virus, emulsified with Freund's complete adjuvant. Twenty-seven days later, blood was collected for the first time, and after another 20 days for the second time. As the titre of the antiserum to Mor turned out to be very low, we injected the same rabbit again intramuscularly with a mixture of 1 ml of the purified virus suspension (0.8 mg ml^{-1}) and 1 ml of Freund's complete adjuvant. After 14 days blood was collected and the titre of the antiserum proved to be satisfactory. Antiserum to W (titre 512) was provided by Dr N.A.M. van Beek, Laboratory of Virology, Wageningen, to NR by Dr O.W. Barnett, to a Nigerian isolate of BlCMV (Nig, titre 256) by Dr H. Huttinga, IPO, Wageningen, and to an isolate of BlCMV from New York (Flo) and to a Moroccan isolate of CAMV (Mor) by Dr D. Gonsalves, Cornell University, Geneva, USA. Henceforth, the antiserum to Mor prepared by us

is designated as-Mor_1, and the one obtained from Dr Gonsalves as-Mor_2.

Antiserum titres were determined by microprecipitation under paraffin oil, using purified virus suspensions. Dilution series of antisera and antigens were prepared with 0.1 M tris (= tris(hydroxmethyl)-aminomethane)-citric acid, pH 8.0). Reactions were recorded after 6 to 21 h at room temperature.

Serological relationships were studied in double sodium dodecyl sulfate (SDS) immunodiffusion tests and in direct enzyme-linked immunosorbent assay (ELISA). SDS-immunodiffusion tests were performed in agar prepared according to Purcifull and Batchelor (1977). The medium contained 0.8% (w/v) Bacto-agar, 1.0% (w/v) NaN_3 and 0.5% (w/v) SDS in deionized water. Purified virus suspensions of 0.5 mg ml^{-1} and infected or uninfected leaf material of cowpea, *N. benthamiana*, or soybean (1 g tissue ground in 2 ml of deionized water or 0.5% (w/v) SDS) were used as antigens.

For use in the double antibody sandwich ELISA, the γ-globulin fractions of the antisera were conjugated with alkaline phosphatase (Clark and Adams, 1977). Coating of the microtiter plates was with 5 μg γ-globulin ml^{-1}. A solution of 0.1 M potassium phosphate + 0.1 M ethylenediamine tetracetic acid (EDTA) adjusted to pH 7.5 was used for extraction and dilution. Healthy and infected leaf tissues of *N. benthamiana* were homogenized in the latter solution at a ratio of 1:20. The final dilution of the conjugated γ-globulin (1 mg ml^{-1}) used was 1:400. Three wells were filled per sample in each plate. The absorbance values at 405 nm were measured with a Titertek Multiskan one h after addition of the substrate (p-nitrophenyl phosphate) and those of sap from healthy plants were subtracted from those of sap from infected plants.

Results

Host-range tests. Out of the 64 species, cultivars and breeding lines tested, Fla infected 23 legumes (19 with symptoms) and 11 non-legumes (5 with symptoms), Ind 28 legumes (23 with symptoms) and 19 non-legumes (6 with symptoms), Mor 21 legumes (20 with symptoms) and 9 non-legumes (8 with symptoms), NR 18 legumes (17 with symptoms) and 7 non-legumes (with symptoms), and W 25 legumes (23 with symptoms) and 17 non-legumes (7 with symptoms) (Tables 1 and 2).

The symptoms on cowpea cv. California Blackeye did not differ appreciably among Fla, Ind, NR and W but they were much more severe with Mor. The symptoms on yardlong bean (*V. unguiculata* ssp. *sesquipedalis*) were similar with Fla, Mor, NR and W but infection was only local and symptomless with Ind. Marked differences were found with the following legumes tested: *Crotalaria juncea* (clear symptoms with Ind and NR, symptomless with Fla, Mor and W), *Cyamopsis tetragonoloba* (clear symptoms with Ind, Mor, NR and W, symptomless with Fla), *G. max* (severe symptoms with Ind, low rates of infection with Fla and W, no infection with Mor and NR), the bean cultivars Noordhollandse Bruine (systemic symptoms with Ind only, latent systemic infection with Fla, no systemic infection with Mor, NR and W), Saxa and The Prince (systemic symptoms with Fla, Ind and NR, no systemic infection with Mor and W), *Trigonella foenum-graecum* (severe symptoms with Ind and Mor, symptomless with Fla, NR and W).

The IITA cowpea breeding lines showed the following marked differences in reactions: TVu 196 (distinct systemic symptoms in all the plants with Fla, NR and W, in

eight out of the 24 plants with Mor, hardly any systemic reaction with Ind), TVu 401 (systemic symptoms in 15 out of the 30 plants inoculated with Ind, in six out of the 28 inoculated with Mor and NR, and in two out of the 40 inoculated with either Fla or W), TVu 1582 and TVu 1593 (distinct systemic symptoms in more than one third of the number of plants inoculated with either Fla, NR or W, vague mottling or symptomless infection with Ind, no infection with Mor), TVu 3273 (no infection with NR, systemic symptoms in less than one fourth of the number of plants inoculated with either Fla or W, mild mosaic in most of the plants with Ind, and severe mosaic in all the plants with Mor), TVu 3433 (no infection with Ind and W, systemic symptoms in six out of the 24 plants with NR, systemic mosaic in all the plants with Fla and Mor).

A problem in evaluating the reactions of the cowpea breeding lines was that plants of the same line did not always show uniform reactions. In some experiments less than 70% of the plants of a susceptible line showed symptoms. Lack of uniformity in the reaction of these cowpea lines has also been observed by Taiwo et al. (1982) who attributed it to either heterogeneity of the lines or to pathogenic variability among the virus isolates. Variation of symptom expression within cowpea cultivars inoculated with BlCMV has also been reported by Collins et al. (1985).

N. benthamiana was the only non-legume with a marked difference in reaction, showing severe leaf deformation (epinasty, curling, narrowing up to 'shoestringing') with W, some leaf deformation (bulging, narrowing) and mottling with Mor, slight epinasty, mild mosaic, mottling and dark-green veinbanding with Fla and Ind, and slight epinasty, mottling and veinal chlorosis with NR (Fig. 1).

Table 3 allows a comparison of the reactions of a selected number of test plant species, cultivars and breeding lines to the five isolates with those induced by BlCMV and CAMV as reported in the literature.

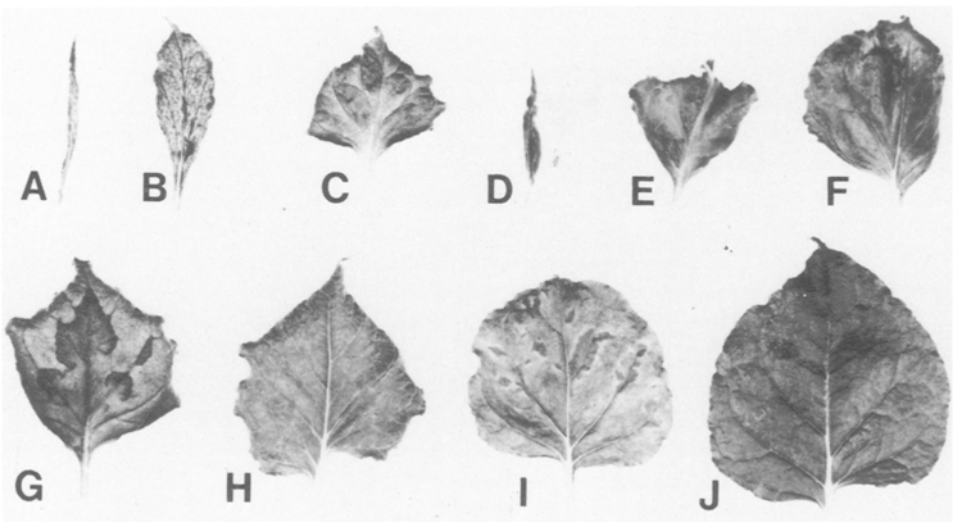

Fig. 1. Leaves of *Nicotiana benthamiana* with deformation (A, B, C and D, E, F,), curling and veinbanding (G), curling and veinal chlorosis (H), and veinbanding (I) after inoculation of the potyvirus isolates W (A, B, C), Mor (D, E, F,), Fla (G), NR (H) and Ind (I); J = healthy control.

Table 1. Reactions of leguminous test plants to five potyvirus isolates from cowpea (Fla, Mor, NR), soybean (Ind), and yard-long bean (W).

Test plants	Fla		Ind		Mor		NR		W	
	local	systemic	local	systemic	local	systemic	local	systemic	local	systemic
Arachis hypogaea	--	--	+	--	--	--	--	--	+	--
Crotalaria juncea	+	+	NL	Mos, VB	--	--	+	LD, Mos	+	+, VB
Cyamopsis tetragonoloba	+	--	CS, NS	VY	CS	Mot, VY	-	Mot	CS	CS, VY
Glycine max	--	-±	CS, NS	LC, Mos, *Mot*, YS	--	--	--	--	--, CS	-, ±
Lens culinaris	--	--	--	--	--	--	--	--	--	--
Mucuna utilis	CS, NS	Mos, Mot	CS	C, Mot	--	--	--	--	CS, NS	C, Mot
Phaseolus vulgaris 'Bataaf'	CS	--	CS	--	NS	--	CS, NS	--	CS	--, +
Phaseolus vulgaris 'Dubbele Witte'	CS	LC, Mos	CS, NS	CS, VN	CS, NS	--	--	--	NS	+, E, LR, VC
Phaseolus vulgaris 'Noordhollandse Bruine'	CS	+	CS	CS, NS	CS, NS	--	CS, NS	--	CS	--
Phaseolus vulgaris 'Pinto	CS	--	CS	--	CS, NS	--	CS	--	NS	--
Phaseolus vulgaris 'Saxa'	CS	CS, NS, VN	CS	C, NS, YS	CS, NS	--	CS, NS	CS	CS, NS	--
Phaseolus vulgaris 'The Prince'	CS	CS, VN	CS	Mos, YS	CS	--	+, C	--	CS, NS	--
Pisum sativum 'Koroza'	--	--	+	+	--	--	--	--	--	--
Pisum sativum 'Rondo'	--	--	+	+	--	--	--	--	--	--
Trifolium incarnatum	--	--	--	--	--, -	--,VY	+	--	--	--
Trifolium pratense	--	--	--	--	--	--	--	--	--	--
Trifolium repens	--	--	--	--	--	--	--	--	--	--
Trifolium subterraneum	•	•	•	•	--	--	--	--	--	--
Trigonella foenum-graecum	+	+	CS, NS	VB, VY	-	VY	--	--	+	+
Vicia faba 'Driemaal Wit'	NS	CS, NS	CS, NS	CS, NS	--,-	--, CS, NS	--,-	--, CS, NS	--, CS, NS	--, NS
Vigna radiata var. radiata	--	--	+	--	--	--	--	--	--	--
Vigna unguiculata ssp. *cylindrica*	CS, YS	LC, Mos, VB	CS	YS	CS, NS	Mos, VY	CS, E	LC, LD, VB	CS, NS	VB, VY
Vigna unguiculata ssp. *sesquipedalis*	-	CS, Mos	+	--	--	CS, Mos	-	Mos, VB	CS, VN	LC, Mos, VB
Vigna unguiculata ssp. *unguiculata* 'California Blackeye'	CS, NS	LD, Mos, Mot, VB, YS	CS, NS	Mos, VB	CS	LC, LD, Mos	CS, NS	LC, VB	CS, NS	Mos, Mot, VB, VY
Vigna unguiculata ssp. *unguiculata* 'Early Red'	--	--	--	--	-	Mos, YS	--	--	--	--

TVu 196	-	CS, Mos	+	+, Mot	--,-	--, Mos	NS, VN	E, VB, YS	E, NS, VN	LD, Mos, VB
TVu 401	--,-	--, +, Mos, VY	--,-	--, Mot, VY	--,-	--, Mos	--,-	--, Mot	--,-	--, Mos
TVu 1582	NS	Mos	CS	+, Mot	--	--	CS, E	E, LC, LD, Mos	CS, VN	Mos, VB, YS
TVu 1593	+	Mos, VY	CS	+	--	--	CS	E, LC, LD, Mos	-, VN	Mos, VB, VN
TVu 2460	•	•	CS	NS	•	•	•	•	+	VC
TVu 2657	--	--	CS	+, Mot	--,-	--, Mos, VY	--	--	--,-, NS	--, Mos
TVu 2740	--,-	--, Mos	+, CS	--	+	+	--	--	--,-	--, Mos
TVu 2845	-	Mos, VB	CS	LC, Mos	CS, VN	LC, LD, VY	CS, VN	E, LC, LD, Mos, VB	CS	LC, VB
TVu 3273	+	--, Mos	+	Mos	CS	Mos	--	--	--,-	--, Mos, VB
TVu 3433	-	Mos	--	--	CS	Mos	--,-	--, YS	--	--

Legend: C = chlorosis; CS = chlorotic spots; E = epinasty; LC = leaf curling; LD = leaf deformation; LR = leaf rolling; Mos = mosaic; Mot = mottling; NL = necrotic lesions; NS = necrotic spots; TVu = IITA breeding lines of *V. unguiculata*; VB = vein banding; VC = veinal chlorosis; VN = veinal necrosis; VY = vein yellowing; YS = yellow spots.
- = no symptoms; -- = no infection; + = symptomless infection; ± = in a few tests a positive result at back inoculations; • = not tested.

Table 2. Reactions of non-leguminous plants to five potyvirus isolates from cowpea (Fla, Mor, NR), soybean (Ind), and yard-long bean (W).

Test plants	Fla		Ind		Mor		NR		W	
	local	systemic	local	systemic	local	systemic	local	systemic	local	systemic
Beta vulgaris 'Groeningia'	--	--	--	--	--	--	--	--	--	--
Capsicum annuum 'Westlandse Zoete'	--	--	-*	-*	--	--	--	--	--	--
Celosia cristata	-*	-*	-*	-*	--	--	--	--	--	+
Chenopodium album	CS, NS	--	CS, NS	--	CS	--	CS	--	CS	--
Chenopodium amaranticolor	NS	--	CS, NS	--	CS, NS	--	CS, NS	--	NS	--
Chenopodium quinoa	CS	--	CS	--	CS	--	CS	--	CS	--
Cucumis sativus 'Lange Gele Tros'	--	--	--	--	--	--	--	--	--	--
Cucurbita pepo 'Verrucosa'	--	--	--	--	--	--	--	--	--	--
Datura stramonium	--	--	--	--	--,-	--,+	--	--	--	--
Gomphrena globosa	+	--	+	--	--, NS	--	NS	+	--, +	--
Helianthus annuus 'Sungold'	+	--	+	--	--	--	--	--	--	--
Lycopersicon esculentum 'Moneymaker'	--	--	--	--	--	--	--	--	--	--
Nicotiana benthamiana	-	LC, VB, VC	Mot	LC, Mos, VB, VC	-	LC, LD, VC	-	LC, Mot, VC	Mot	LC, LD, LN, Mot, VC
Nicotiana clevelandii	+	+	-	Mot	-	Mot	--	--	Mot	Mot
Nicotiana debneyi	+	--	+	--	•	•	•	•	+	--
Nicotiana glutinosa	--	--	--	--	--	--	--	--	+	--
Nicotiana rustica	--	--	+	--	--	--	--	--	+	--
Nicotiana tabacum 'Samsun NN'	--	--	+	--	--	--	--	--	+	--
Nicotiana tabacum 'White Burley'	--	--	+	--	--	--	--	--	Mot	--
Nicotiana tabacum 'Xanthi-nc'	--	--	+	--	--	--	--	--	+	--
Ocimum basilicum	--	--	+	--	--	--	--	--	--	--
Petunia hybrida	--	--	+	--	--	--	+	--	--, +	--
Physalis alkekengi	--	--	--	+	--	--	--	--	--	--
Physalis floridana	--	--	+	+	--	--	--	--	--	--
Solanum melongena 'Lange Violette'	--	--	--	--	--	--	--	--	--	--
Spinacia oleracea	+	+	+	+	--	--	--	--	--, +	--
Tetragonia expansa	CS, NS	+	CS	--	CS	--	CS	--	CS	--
Torenia fournieri	+	--	+	+	+	--, VN	--	--	+	--
Zinnia elegans 'Persian Carpet'	--	--	--	--	--	--	--	--	--	--

Legend: LN = leaf narrowing; -* = no symptoms, no check by back inoculation. For the other legends see Table 1.

Properties in crude sap. The dilution endpoint of Fla, Mor, NR and W was 10^{-3} to 10^{-4}; that of Ind was 10^{-4} to 10^{-5}. The inactivation temperature of Fla, NR and W was similar, viz. 60 to 70 °C, whereas that of Ind and Mor was slightly lower (50 to 60 °C). The isolate Ind was inactivated between 24 and 48 h, both Fla and W between 72 and 96 h, and NR and Mor between 96 and 120 h and 144 and 168 h, respectively.

Transmission by aphids. *Myzus persicae* transmitted the five isolates as follows. From cowpea to cowpea: Fla, 6 out of 10 plants; Ind, none out of 10 plants; Mor, 8 out of 11 plants; NR, 7 out of 10 plants; and W, 6 out of 10 plants. From *N. benthamiana* to *N. benthamiana*: Fla and NR, 2 out of 6 plants in both experiments; Ind, 1 out of 5 plants in one experiment and 2 out of 6 plants in the second experiment; W, 5 out of 6 plants in one, and all six in the other experiment; and most remarkably, Mor none of the six plants in either experiment.

Purification. Purified suspensions of the five isolates had absorption maxima at 260 nm and minima at about 245 nm. The A_{max}/A_{min} absorption ratios were 1.17 for Fla, 1.09-1.12 for Ind, 1.15 for Mor, 1.05 for NR, and 1.07-1.21 for W. Virus yields per 100 g leaf material averaged from five purifications each about 0.7 mg, 1 mg, 1-2 mg, 1 mg, and 1-3 mg for Fla, Ind, Mor, NR and W, respectively.

Polyacrylamide gel electrophoresis of the coat protein of each of the five isolates revealed two to three bands, the most prominent one being composed of the slowest migrating polypeptide. Molecular masses of the proteins in these bands ranged from 34 000 to 33 000 Da, 32 000 to 31 000 Da and 29 000 to 28 000 Da for all five isolates (values obtained in two independent experiments with different virus preparations).

Electron microscopy. In ultrathin sections of leaves of plants of *N. benthamiana*, systemically infected with each of the five isolates, we observed pinwheels typical of those of subdivision I (scrolls attached to the central portion of cylindrical inclusions) according to the classification of inclusions induced by potyviruses (Edwardson, 1974).

Serology. The antisera to Fla and Ind had titres of 256 and 128, respectively, whereas the titre of as-Mor_1 was 512.

Comparison of the isolates in immunodiffusion tests. The antisera to Fla, Ind, Mor, NR and W reacted with clear precipitin lines in the immunodiffusion test, with both purified virus suspensions and sap from infected plants, but not from uninfected ones. The antisera to Nig and W gave a hardly visible reaction and antisera to Flo and Mor_2 gave a strong reaction with sap from uninfected plants. However, these non-specific reaction lines could easily be distinguished from those resulting from specific reactions.

Reciprocal immunodiffusion tests with Fla, NR and W antisera showed that they were serologically identical (Table 4). With Flo and Nig antisera, the precipitin lines with Fla and W were as strong as in case of the homologous reactions of Fla and W. The isolate Ind proved to be serologically related but not identical to the isolates Fla, Mor, NR and W (Table 4) with spurs extending beyond the heterologous reaction lines, as shown in Fig. 2 with as-Mor_2 (A) and as-W (C, D). Similar results were obtained in reciprocal tests with as-Ind. Antiserum to isolate Mor (both as-Mor_1 and as-Mor_2)

Table 3. Reactions of test plants to five potyvirus isolates from cowpea (Fla, Mor, NR), soybean (Ind) and yard-long bean (W), compared with those of blackeye cowpea mosaic virus (BlCMV) and cowpea aphid-borne mosaic virus (CAMV) mentioned in the literature.

Test plants	Fla		Ind		Mor		NR		W		BlCMV[†1,2,3,4]		CAMV[†3,5,6]	
Legumes														
Glycine max	-	-,s	L	S	-	-	-	-	-,L	s[a]	l	-[1]	l	S[6]
											l	S[2]		
Trifolium repens	-	-	-	-	-	-	-	-	-	-	-	-[4]	l	S[5]
													-	-[6]
Vicia faba	L	S	L	S	-,l	S[a]	-,l	S[a]	-,L	S[a]	l	s[1]	L	S[5]
											-	-[2,4]	-	-[6]
Vigna unguiculata 'Blackeye'	L	S	L	S	L	S	L	S	L	S	L	S[1]	L	S[6]
TVu 196	l	S	l	S[a]	-,l	S[a]	L	S	L	S	l	S[3]	-	S[a,3]
TVu 401	-,l	S[a]	-,l	-,S	-,l	S[a]	-,l	S[a]	-,l	S[a]	l	S[3]	-	S[a,3]
TVu 1582	L	S	L	S[a]	-	-	L	S	L	S	l	S[3]	-*	-*,3
TVu 1593	-,l	S[a]	L	s	-	-	L	S	l,L	S	l	S[3]	-	S[a,3]
TVu 2460	•	•	L	S	•	•	•	•	l	S	l	S[3]	-	S[a,3]
TVu 2657	-	-	L	S[a]	-,l	S[a]	-	-	-,l,L	S[a]	l	S[3]	l	S[3]
TVu 2740	-,l	S[a]	l,L	-	l	s	-	-	-,l	S[a]	-*	-*,3	l	S[3]
TVu 2845	l	S	L	S	L	S	L	S	L	S	-*	-*,3	l	S[3]
TVu 3273	-,l	S[a]	l	S[a]	L	S	-	-	-,l	S[a]	-*	-*,3	l	S[3]
TVu 3433	-,l	S[a]	-	-	L	S	-,l	S[a]	-	-	-*	-*,3	l	S[3]
Non-legumes														
Capsicum annuum	-	-	-*	-*	-	-	-	-	-	-	-	-[2]	-	-[6]
Chenopodium amaranticolor	L	-	L	-	L	-	L	-	L	-	L	-[2]	L	-[5,6]
Chenopodium quinoa	L	-	L	-	L	-	L	-	L	-	L	-[2]	L	-[5,6]
Cucumis sativus	-	-	-	-	-	-	-	-	-	-	-	-[2,4]	-	-[5]
													l	S[6]

Cucurbita pepo	-	-	-	-	-	-	-	-	-	-	-	-[2,4]	l	S[6]
Gomphrena globosa	l	-	l	-	l,L	-	L	s	-,l	-	-	-[4]	L	-[5,6]
Lycopersicon esculentum	-	-	-	-	-	-	-	-	-	-	-	-[1]	-	-[6]
Nicotiana glutinosa	-	-	-	-	-	-	-	-	l	-	-	-[1]	l	-[5]
													-	-[6]
Nicotiana tabacum 'White Burley'	-	-	-	-	-	-	-	-	L	-	-	-[1,4]	L	-[5,6]
Ocimum basilicum	-	-	l	-	-	-	-	-	-	-	L	-[2]	L	-[6]

Legend: L = visible local infection; S = visible systemic infection; l = latent local infection; s = latent systemic infection; TVu = IITA breeding lines of *V. unguiculata*; - = no infection as tested by back inoculation; -* = no symptoms, no check by back inoculation; • = not tested; [a] = reaction in less than half the number of plants inoculated.

[†] = references; [1] = Anderson, 1955; [2] = Lima et al., 1979; [3] = Taiwo et al., 1982; [4] = Tsuchizaki et al., 1970; [5] = Bos, 1970; [6] = Lovisolo & Conti, 1966.

Table 4. Reactions of three potyvirus isolates from cowpea (Fla, Mor, NR), one from soybean (Ind) and one from yard-long bean (W) to homologous and heterologous antisera in sodium dodecyl sulfate immunodiffusion tests (ID) and in direct enzyme-linked immunosorbent assay (ELISA).

Antisera	Antigens[1]									
	Fla		Ind		Mor		NR		W	
	ID	ELISA[2]	ID	ELISA[2]	ID	ELISA[2]	ID	ELISA[2]	ID	ELISA[2]
Fla	○○○	100	○○	1	○○○	84	○○○	96	○○○	81
Ind	○○	17	○○○	100	○○	12	○○	13	○○	12
Mor_1[3]	○	1	○	1	○○○	100	○	0	○	1
Mor_2	○○		○○		○○○		○○		○○	
NR	○○○	96	○	18	○○○	100	○○○	100	○○○	100
W	○○○	100	○○	3	○○○	88	○○○	100	○○○	100

[1] For ID 1 g leaf tissue of *Glycine max*, *Nicotiana benthamiana* or *Vigna unguiculata* was ground in 2 ml of deionized water; for ELISA infected leaves of *N. benthamiana* were homogenized in 20 ml 0.1 M potassium phosphate + 0.1 M ethylenediamine tetracetic acid, pH 7.5.

[2] Absorbance value at 405 nm as percentage of that of the homologous reaction.

[3] Mor_1 prepared by us; Mor_2 obtained from Dr Gonsalves.

○○○ = Reaction of homology or identity; ○○ = Strong heterologous reaction with spur formation; ○ = Weak heterologous reaction with spur formation.

gave weak but distinct reactions with suspensions of Fla, Ind, NR and W, the reactions being stronger for as-Mor_2. The precipitin lines coalesced when Fla, Ind, NR and W were in adjacent wells, as can be seen in Fig. 2A with Ind and NR. In the reciprocal tests, however, Fla, Mor, NR and W were serologically identical, as shown by coalescing precipitin lines which formed spurs with Ind only. This is partly illustrated by Fig. 2B with as-Fla against Mor, NR and W as antigens.

Comparison of the isolates in ELISA. Results of ELISA are summarized in Table 4. The isolates Fla, NR and W reacted strongly to their homologous and heterologous antisera, but much less to antiserum to Ind and not at all to that to Mor. However, when Mor antigen was tested against antisera to Fla, NR and W there were strong reactions, hardly distinguishable from the homologous ones. Antisera to Fla, Mor, NR and W reacted very weakly to Ind, and the reactions were similar in reciprocal tests.

The ELISA results corroborated those obtained in immunodiffusion tests.

Discussion

The results of host-range studies and serological tests showed that the isolates Fla, Ind, Mor, NR and W were closely related mutually and to BlCMV and CAMV of the literature (Anderson, 1955; Bock and Conti, 1974; Bos, 1970; Lima et al., 1979; Lovisolo and Conti, 1966; Taiwo et al., 1982).

The five isolates did not differ much in their dilution endpoints and thermal inact-

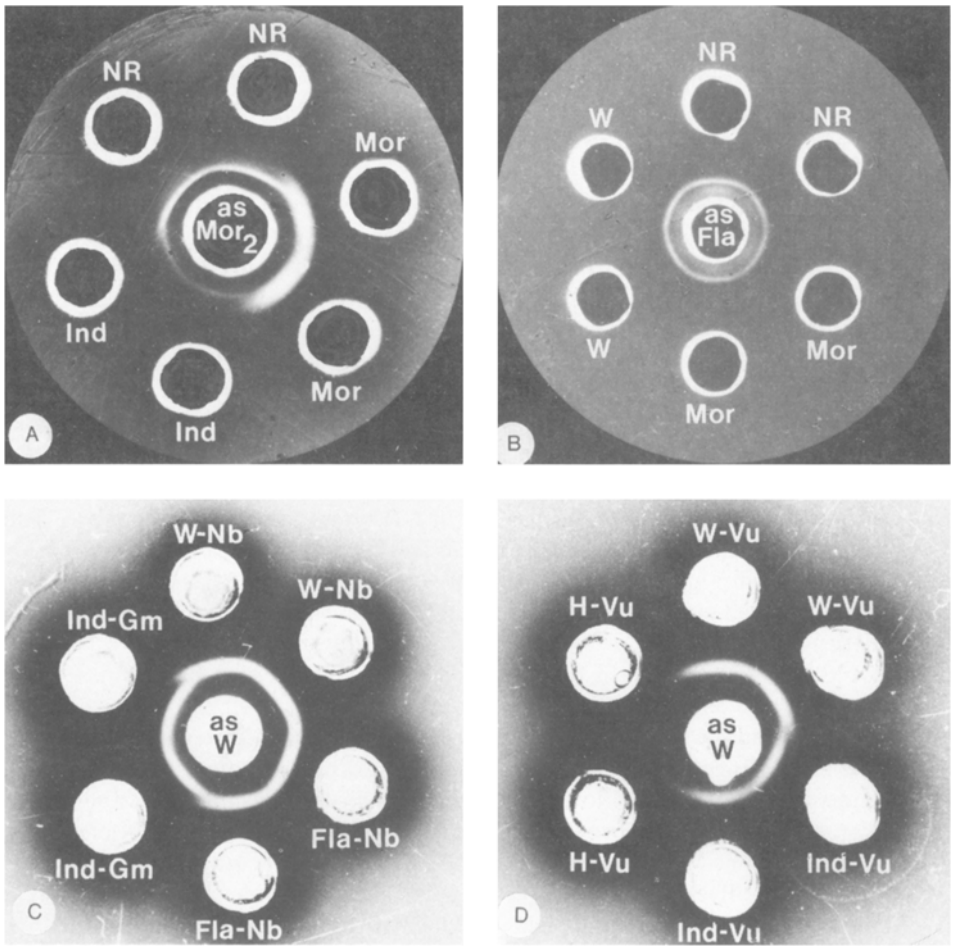

Fig. 2A-D. Immunodiffusion tests with the potyvirus isolates Fla, Ind, Mor, NR and W, either in sap from infected plants of *Glycine max* (Ind-Gm), *Nicotiana benthamiana* (Fla-Nb, W-Nb) and *Vigna unguiculata* (Ind-Vu), or as purified suspension (Ind, Mor, NR and W). H-Vu = sap from uninfected plants of *V. unguiculata*; as Fla, as Mor_2 and as W are antisera to Fla, Mor and W, respectively.

ivation points among themselves and from the BlCMV and CAMV isolates described in the literature (Bock and Conti, 1974; Lima et al., 1979; Lovisolo and Conti, 1966; Taiwo et al., 1982). More differences were found in the longevity in vitro, with values ranging from 24 to 48 h (Ind), 72 to 120 h (Fla, NR and W), and 144 to 168 h (Mor). The values mentioned in the literature for BlCMV are 48 h (Lima et al., 1979) and 24 to 48 h (Taiwo et al., 1982), and for CAMV 24 to 48 h (Lovisolo and Conti, 1966) and 120 to 125 h (Bock and Conti, 1974). However, such data are known to vary and to be of little value to the identification of viruses (Francki, 1980).

The pinwheel inclusions observed were similar to those for BlCMV (Christie and

Edwardson, 1977; Edwardson et al., 1972; Lima et al., 1979) and to those of CAMV (Lima et al., 1981). The possibility that isolate Ind was SMV was excluded on the basis of reactions of test plants and the type of inclusions. The former differed very much from those mentioned for SMV (Bos, 1972), whereas the inclusions were typical of those of subdivision I and not of subdivision III to which those of SMV belong (Edwardson, 1974).

The molecular masses of the coat protein of the five virus isolates and the capsid protein heterogeneity were also comparable to those reported for BlCMV and CAMV (Taiwo et al., 1982), as well as to those of bean common mosaic virus (BCMV), bean yellow mosaic virus (BYMV) and other potyviruses (Hollings and Brunt, 1981).

Serologically, the isolates Ind and Mor were each different from the other isolates and from each other as shown by the spurs of the homologous diffusion lines in agar over those of the heterologous lines and by the results in ELISA.

There was a striking difference in the results of reciprocal tests with Mor on the one hand and Fla, NR and W on the other. Both in immunodiffusion tests and in ELISA the four virus isolates were identical when Mor was reacted with as-Fla, as-NR or as-W. In reciprocal tests, however, with either Fla or NR or W and as-Mor there was clear spur formation in immunodiffusion tests and no reaction in ELISA. Contamination of Mor antigen with either Fla or NR or W can be excluded as as-Mor_1 had been prepared to purified virus from the same batch which was used as antigen in the immunodiffusion tests.

We could not assign any of the five isolates categorically to either BlCMV or CAMV on the basis of the results obtained.

The literature on the distinction between these two viruses also remains confusing. Anderson (1955) introduced the name blackeye cowpea mosaic virus for a virus causing mosaic in cowpea in Florida in USA. This virus, transmitted by aphids and by seed, was considered by Corbett (1957) to be a strain of BYMV, but differences in cylindrical inclusions led Edwardson et al. (1972) later to conclude that BlCMV and BYMV were two different viruses. The original virus of Anderson was lost, but a BlCMV has been reisolated from field-grown cowpea in Florida. Lima et al. (1979) have further characterized the latter virus and confirmed serologically the difference between BlCMV and BYMV. They also found that their BlCMV was serologically related to, but distinct from BCMV, CAMV, dasheen mosaic virus, lettuce mosaic virus, tobacco etch virus, potato virus Y, SMV and watermelon mosaic virus 2.

In 1966, Lovisolo and Conti published about an aphid-transmitted, seed-borne potyvirus from mosaic-diseased cowpea plants in northern Italy. They found it to be serologically related to BCMV, but as it was distinct from the cowpea strain of BYMV, the name given by Corbett (1957) to Anderson's BlCMV, they tentatively named their virus CAMV. The characterization of CAMV and its distinction from some other potyviruses was later corroborated by Bos (1970). Afterwards, Bock (1973) described CAMV isolated from infected cowpea in East Africa and distinguished three strains on the basis of host range and serology. His East African isolates were serologically related to BCMV, but not to BYMV. He arrived at the conclusion that at least one of his East African strains closely resembled the European type isolate described by Lovisolo and Conti (1966). However, no reciprocal serological tests were performed between the East African isolates and the European type strain of CAMV, so that identity between the two viruses was not established. Later, Bock and Conti (1974)

published a description of the virus based on data obtained with the European type isolate and the East African strains. *G. max*, *Ocimum basilicum* and *Petunia hybrida* were mentioned as diagnostic species; serological relationship to BCMV, but not to BYMV, was reported and possible relationship to or synonymy with BlCMV was not excluded. Like Anderson's BlCMV, the original isolate of CAMV from northern Italy has also been lost, and no antiserum to it is available anymore. In contrast to BlCMV having been reisolated (Lima et al., 1979), there are no reports that CAMV has been reisolated from cowpea in northern Italy. This has ruled out the possibility of valid comparisons between the original CAMV and any other unidentified virus resembling CAMV.

Lima et al. (1979) have suggested that BlCMV and CAMV should be regarded as distinct members of the potyvirus group, because of differences in host range and a distant serological relationship between an isolate of CAMV from Morocco, described by Fischer and Lockhart (1976), and BlCMV reisolated from cowpea in Florida. Taiwo and Gonsalves (1982) thereafter serologically grouped isolates from Florida and New York, as well as isolates from Kenya and Nigeria, originally considered to be CAMV, as BlCMV, and the isolate from Morocco (Fischer and Lockhart, 1976) and a previously undescribed isolate from Cyprus, as CAMV. In immunoelectron microscopy, homologous and heterologous decoration within the BlCMV group of isolates was heavy and the same held for the CAMV group of two isolates. The latter two isolates were moderately decorated with antisera to viruses of the BlCMV group, but reciprocal decoration did not occur. A distinction between BlCMV and CAMV isolates was corroborated by Taiwo et al. (1982), using a series of IITA TVu breeding lines of cowpea with differential resistance to BlCMV and CAMV. However, they found that the isolates were very similar in physico-chemical properties. Serology has also been the main criterion for Lima et al. (1981) and Lin et al. (1981) to distinguish between BlCMV and CAMV found in Brazil. Iwaki et al. (1975) reported on the occurrence of a potyvirus in cowpea in Indonesia. They identified the virus as CAMV according to a proposal of Tsuchizaki et al. (1970) who described a similar virus from Tokyo (Japan). The latter authors, however, later revised their opinion on the basis of results in serological tests, and reached the conclusion that the Tokyo isolate should be regarded as BlCMV instead of CAMV (Tsuchizaki et al., 1984).

Our results raise doubts about the existence of BlCMV and CAMV as two distinct viruses. Some test plants showed reactions to all five isolates similar to those attributable to CAMV, as described by Lovisolo and Conti (1966), whereas the reactions on other test plants resembled those caused by BlCMV. On the cowpea differential lines of Taiwo et al. (1982), Fla, NR and W isolates more closely resembled BlCMV, but reactions were also obtained on lines which according to them should have been only with CAMV. The Ind isolate differed from both BlCMV and CAMV. We must point out that Taiwo et al. (1982) tested each line with very small numbers of plants (in some experiments only three plants per line) while in our studies 30 to 50 plants of each line were used.

Serologically, our isolate W was identical to isolates Fla and NR, supposed to be BlCMV, and the isolates Ind and Mor appeared to be deviating strains. Serological differences, however, should be regarded with caution, especially with potyviruses, since antigenic determinants may differ, depending on the way the virus has been purified or stored (Lima et al., 1979). Taiwo and Gonsalves (1982) have shown that antisera to

one virus may differ qualitatively: Antiserum to BlCMV prepared by Lima et al. (1979) reacted strongly with the Moroccan CAMV, whereas the antiserum they had prepared to BlCMV did not give any reaction with it.

Important are the relationships of both viruses to BCMV as first reported for CAMV by Lovisolo and Conti (1966). Fischer and Lockhart (1976), however, did not get any reaction with their Moroccan isolate of CAMV and antiserum to BCMV. Lima et al. (1979) reported a distant relationship between BlCMV and an isolate of BCMV. Taiwo and Gonsalves (1982) found a close relationship between an isolate of BCMV and four isolates of BlCMV, and a distant relationship between the former virus and isolates of CAMV. Two isolates of BlCMV from *Phaseolus* bean in Lebanon gave strong reactions with both antiserum to BlCMV and that to the NY 15 isolate of BCMV, but a weak reaction with antisera to the BCMV isolates NL 3 and NL 5 (Makkouk et al., 1986).

Several strains of BCMV have been identified by their characteristic interactions with a selected series of differential *Phaseolus*-bean cultivars. All strains produce mosaic on one or more cultivars possessing recessive alleles of a necrosis gene I. Some strains also induce necrosis ('black root disease') on bean cultivars having dominant alleles of the necrosis gene (Drijfhout, 1978). Research in progress at Prosser, USA, points to a serological distinction between the latter group of strains (serogroup A) and the group of non-necrosis inducing strains (serogroup B), and also to the detectability of BlCMV with B antisera and of CAMV with A antisera (G.I. Mink, personal communication, 1986). This might suggest an essential difference between the two viruses under discussion. However, here also, the differences are not that rigid since BCMV-NL 8 biologically classifies with the 'necrotic' strains, but serologically fits with the B group (G.I. Mink, personal communication, 1986). Because of this, and since such serologically differing biotypes of BCMV are still considered strains of one virus, whereas the differences found by us between the alleged isolates of BlCMV and CAMV are even smaller, especially in pathogenicity, BlCMV and CAMV have to be considered synonyms. In this, BlCMV has the obvious chronological priority. Moreover, it has been well characterized (Edwardson et al., 1972; Lima et al., 1979; Taiwo and Gonsalves, 1982; Taiwo et al., 1982; Zettler and Evans, 1972) on the basis of a neotype isolated from the same region as Anderson's (Anderson, 1955) and with apparently similar properties. Recently, an official description of BlCMV has been made by Purcifull and Gonsalves (1985). In contrast, no type strain and antiserum of CAMV are available anymore nor has this virus been reisolated in Italy, where it was first described. The 'Description' of CAMV (Bock and Conti, 1974) most likely pertains to the original CAMV of Lovisolo and Conti (1966) and to Bock's Kenyan virus (Bock, 1973) which has later been partly reidentified as BlCMV (Taiwo and Gonsalves, 1982).

The confusion described here reemphasizes the absolute necessity of preservation of type material of plant viruses for future reference and completion of the characterization (Bos, 1964).

In the light of our results and discussion we consider all isolates studied here to be isolates of one and the same virus, viz. BlCMV, with Fla, NR and W to be similar and Ind and Mor to be distinct strains.

The International Committee on the Taxonomy of Viruses have considered BlCMV and CAMV as two different members of the potyvirus group (Matthews, 1982), but this decision requires reconsideration in view of our findings and suggestion.

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Samenvatting

Identificatie van 'blackeye cowpea mosaic virus' uit genenmateriaal van Vigna unguiculata ssp. sesquipedalis en uit sojaboon, en de relaties tussen 'blackeye cowpea mosaic virus' en 'cowpea aphid-borne mosaic virus'

Twee potyvirussen, de een in Nederland ingevoerd met genenmateriaal van *Vigna unguiculata* ssp. *sesquipedalis* en de ander uit planten van sojaboon (*Glycine max*) in Indonesië, werden vergeleken met twee islolaten van 'blackeye cowpea mosiac virus' (BlCMV) en een Marokkaans isolaat van 'cowpea aphid-borne mosaic virus' (CAMV). Op grond van waardplantenreeksen, symptomen en serologie stellen de auteurs voor om alle vijf isolaten te beschouwen als BlCMV. Gebaseerd op de verkregen resultaten en een kritische beschouwing van de literatuur wordt de aanbeveling gedaan om de naam CAMV te laten vallen ten gunste van BlCMV.

References

Anderson, C.W., 1955. *Vigna* and *Crotalaria* viruses in Florida. II. Notations concerning cowpea mosaic virus (*Marmor vignae*). Plant Disease Reporter 39: 349-353.

Bock, K.R., 1973. East African strains of cowpea aphid-borne mosaic virus. Annals of Applied Biology 74: 75-83.

Bock, K.R. & Conti, M., 1974. Cowpea aphid-borne mosaic virus no. 134. In: Descriptions of plant viruses. Commonwealth Mycological Institute, Association of Applied Biologists, Kew, Surrey, England, 4 pp.

Bos, L., 1964. Tentative list of viruses reported from naturally infected leguminous plants. Netherlands Journal of Plant Pathology 70: 161-174.

Bos, L., 1970. The identification of three new viruses isolated from *Wisteria* and *Pisum* in the Netherlands, and the problem of variation within the potato virus Y group. Netherlands Journal of Plant Pathology 76: 8-46.

Bos, L., 1972. Soybean mosaic virus no. 93. In: Descriptions of plant viruses. Commonwealth Mycological Institute, Association of Applied Biologists, Kew, Surrey, England, 4 pp.

Christie, R.G. & Edwardson, J.R., 1977. Light and electron microscopy of plant virus inclusions. Florida Agricultural Experiment Stations Monograph Series 9. Gainesville, 155 pp.

Clark, M.F. & Adams, A.N., 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34: 475-483.

Collins, M.H., Witcher, W. & Barnett, O.W., 1985. Reactions of 16 cowpea cultivars to six viruses. Plant Disease 69: 18-20.

Corbett, M.K., 1957. Serological and morphological relationships of plant viruses. Florida Agricultural Experiment Stations Annual Report 1956: 117-118.

Dijkstra, J., Clement, Y. & Lohuis, H., 1985. Characterization of a carlavirus from dandelion (*Taraxacum officinale*). Netherlands Journal of Plant Pathology 91: 77-92.

Drijfhout, E., 1978. Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. Centre for Agricultural Publishing and Documentation, Wageningen, 98 pp.
Edwardson, J.R., 1974. Some properties of the potato virus Y-group. Florida Agricultural Experiment Stations Monograph Series 4. Gainesville. 398 pp.
Edwardson, J.R., Zettler, F.W., Christie, R.G. & Evans, I.R., 1972. A cytological comparison of inclusions as a basis for distinguishing two filamentous legume viruses. Journal of General Virology 15: 113-118.
Fischer, H.U. & Lockhart, B.E.L., 1976. A strain of cowpea aphid-borne mosaic virus isolated from cowpea in Morocco. Phytopathologische Zeitschrift 85: 43-48.
Francki, R.I.B., 1980. Limited value of the thermal inactivation point, longevity *in vitro* and dilution end-point as criteria for the characterization, identification and classification of plant viruses. Intervirology 13: 91-98.
Hollings, M. & Brunt, A.A., 1981. Potyviruses. In: E. Kurstak (Ed.), Handbook of plant virus infections. Comparative diagnosis. Elsevier/North Holland Biomedical Press, Amsterdam-New York-Oxford, p. 731-807.
Iwaki, M., Roechan, M. & Tantera, D.M., 1975. Virus diseases of legume plants in Indonesia. 1. Cowpea aphid-borne mosaic virus. Contributions of the Central Research Institute for Agriculture, Bogor 13: 14 pp.
Jayasinghe, U. & Dijkstra, J., 1979. *Hippeastrum* mosaic virus and another filamentous virus in *Eucharis grandiflora*. Netherlands Journal of Plant Pathology 85: 47-65.
Lima, J.A.A., Oliveira, F.M.E.W., Kitajima, E.W. & Lima, M.G.A., 1981. Propriedades biológicas, citológicas e sorológicas de um potyvirus isolado de feijão-de-corda no Ceará. Fitopatologia Brasileira 6: 205-216.
Lima, J.A.A., Purcifull, D.E. & Hiebert, E., 1979. Purification, partial characterization, and serology of blackeye cowpea mosaic virus. Phytopathology 69: 1252-1258.
Lin, M.T., Kitajima, E.W. & Rios, G.P., 1981. Serological identification of several cowpea viruses in Central Brazil. Fitopatologia Brasileira 6: 73-85.
Lovisolo, O. & Conti, M., 1966. Identification of an aphid-transmitted cowpea mosaic virus. Netherlands Journal of Plant Pathology 72: 265-269.
Makkouk, K.M., Lesemann, D.E., Vetten, H.J. & Azzam, O.I., 1986. Host range and serological properties of two potyvirus isolates from *Phaseolus vulgaris* in Lebanon. Tropical Agriculture Research Series (Japan) 19: 187-194.
Matthews, R.E.F., 1982. Classification and nomenclature of viruses. S. Karger, New York, 199 pp.
Murphy, J.F., Barnett, O.W. & Witcher, W., 1987. Characterization of a blackeye cowpea mosaic virus strain from South Carolina. Plant Disease 71: 243-248.
Purcifull, D.E., 1966. Some properties of tobacco etch virus and its alkaline degradation products. Virology 29: 8-14.
Purcifull, D.E. & Batchelor, D.L., 1977. Immunodiffusion tests with sodium dodecylsulfate (SDS)-treated plant viruses and plant viral inclusions. Florida Agricultural Experiment Stations Bulletin 788 (Technical). Gainesville, 39 pp.
Purcifull, D.E. & Gonsalves, D., 1985. Blackeye cowpea mosaic virus no. 305. In: Descriptions of plant viruses. Commonwealth Mycological Institute, Association of Applied Biologists, Kew, Surrey, England, 5 pp.
Taiwo, M.A. & Gonsalves, D., 1982. Serological grouping of isolates of blackeye cowpea mosaic virus and cowpea aphidborne mosaic viruses. Phytopathology 72: 583-589.
Taiwo, M.A., Gonsalves, D., Provvidenti, R. & Thurston, H.D., 1982. Partial characterization and grouping of isolates of blackeye cowpea mosaic and cowpea aphidborne mosaic viruses. Phytopathology 72: 590-596.
Tsuchizaki, T., Senboku, T., Iwaki, M., Pholauporn, S., Srithongchi, W., Deema, N. & Ching, A.O., 1984. Blackeye cowpea mosaic virus from Asparagus bean (*Vigna sesquipedalis*) in

Thailand and Malaysia, and their relationships to a Japanese isolate. Annals of the Phytopathological Society of Japan 50: 461-468.

Tsuchizaki, T., Yora, K. & Asuyama, H., 1970. The viruses causing mosaic of cowpea and Azuki bean, and their transmissibility through seeds. Annals of the Phytopathological Society of Japan 36: 112-120.

Zettler, F.W. & Evans, I.R., 1972. Blackeye cowpea mosaic virus in Florida: Host range and incidence in certified cowpea seed. Proceedings of the Florida State Horticultural Society 85: 99-101.