HOST RANGE AND SEROLOGICAL PROPERTIES OF TWO POTYVIRUS ISOLATES FROM *PHASEOLUS VULGARIS* IN LEBANON

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ABSTRACT

Two virus isolates obrained from *Phaseolus vulgaris* in Lebanon were identified as potyviruses with close serological relationship to each other, to blackeye cowpea mosaic virus, to the NY 15 isolate of bean common mosaic virus (BCMV-NY 15) and to azuki bean mosaic virus (AzMV). Both isolates infected six plant species belonging to three families. The two isolates were differentiated from each other by their reaction on six IITA-TVu cowpea lines as well as on seven *P. vulgaris* cultivars. In double antibody sandwich ELISA both virus antigens strongly reacted with BlCMV, BCMV-NY 15 and AzMV antisera and weakly with antisera to cowpea aphid-borne mosaic virus (CAMV), bean yellow mosaic virus (BYMV) and some isolates of BCMV. In reciprocal ELISA tests, antisera to our two virus isolates reacted strongly with BlCMV and BCMV-Ny 15. ISEM tests confirmed the ELISA results, giving strong reactions when both isolates were tested with antisera to BlCMV, BCMV-NY 15 and AzMV.

Introduction

Legumes are known to be suceptible to a large number of viruses. In the Near East bean common mosaic virus (BCMV), bean yellow mosaic virus (BYMV) and cucumber mosaic virus (CMV) have been isolated from *Phaseolus vulgaris* (Haddad, 1983; Lockhart and Fisher, 1974; Mazyad *et al.*, 1974; Nienhaus and Saad, 1967; Omar *et al.*, 1979; Rudolph and Baykal, 1977).

In 1982 we isolated two potyviruses from *P. vulgaris* plants in Lebanon. Field symptoms were essentially mosaic and puckering of the leaves with reduced growth. Our attempts to characterize these two bean isolates raise questions about the relatedness of potyviruses to each other and whether strains of some potyviruses are eligible to be considered as separate viruses or whether some of the already named potyviruses should be grouped together as one virus. This is an unresolved problem in plant virus taxonomy, particularly in the classification of potyviruses. In this study we used host reaction, ELISA and ISEM to evaluate the relatedness of our two bean isolates to other legume potyviruses.

Materials and methods

1 Virus source and propagation

The two virus isolates used in this study were isolated from infected French bean plants collected from the coastal area north of Beirut in 1982. The isolates were maintained in *P. vulgaris,* 'Sutter Pink', and designated 52-82 and 53-82.

2 Host range

Virus isolates were inoculated to 33 plant species representing 10 different families. The temperature in the greenhouse where the test plants were kept ranged rom 20 to 30°C. Infected

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bean leaves were triturated with a mortar and pestle in 0.01M phosphate buffer, pH 7.2, mixed with celite, and inoculated to the test plants. To detect latent infections, plants showing no symptoms were assayed for the presence of the virus by the enzyme-linked immunosorbent assay (ELISA) three weeks after inoculation. Six cowpea lines (IITA-TVu) kindly provided by Dr. L. Bos, The Netherlands and seven bean cultivars representing the international set of BCMV differentials were included in this study.

3 Virus purification and antiserum production

Virus isolates were propagated either in *Nicotiana benthamiana* or in *P. vulgaris* 'Saxa' and purified essentially by the method of Lisa *et al.*, (1981). Following the first high-speed centrifugation and resuspension of pellets in 0.05M sodium citrate buffer (SC), pH 7.5, however, no low-speed centrifugations and no sucrose gradient centrifugation were applied. The resuspended virus was immediately subjected to quasi-isopycnic centrifugation by adding 400 mg/ml CsCl and centrifugation in a Beckman SW 55 T rotor at 35,000 rpm for 15-17 h at 10°C. The virus band was collected from the gradients using a peristaltic pump, diluted with 4-5 vol of SC and sedimented in a Beckman 30 rotor at 28,000 rpm for 4 h. The virus pellet was resuspended in 10-15 ml SC and recentrifuged in CsCl using the conditions mentioned above. After sedimentation of the virus by ultracentrifugation the purity of the virus preparations was assessed by electron microscopy. If phytoferritin and other contaminants were detected, the virus was subjected to a third cycle of quasi-isopycnic and high speed centrifugation. Thereafter virus preparations always appeared to be devoid of contaminating material. Virus yields from *N. benthamiana* and *P. vulgaris* were in the range of 5-10 and 30-50 mg/kg leaf material, respectively.

Rabbits were given two intramuscular injections of 5mg virus at weekly intervals and one additional injection about 4 weeks after the second injection. Freund's complete adjuvant (Difco) was used for the first injection and incomplete adjuvant used for the second and third. Bleedings were taken weekly starting three weeks after the first injection.

4 Electron microscopy

Virus particles were visualized from crude extracts of infected leaves or from purified preparations after absorption to carbon-Pioloform-coated copper grids, and negative staining with 2% aqueous uranyl acetate (UA). A Zeiss EM 10 c electron microscope was used. Particle length was measured on Ua-stained preparations in an electron microscope equipped with a Zeiss Morphomat 30 image-analysing system. The magnification (50,000 x) was calibrated with a diffraction grating replica with a periodicity of 463 nm.

Immunosorbent electron microscopy (ISEM) was used as described previously (Lesemann, 1982). Carbon-Pioloform-coated nickel grids were coated with a solution of 10 μg/ml of protein A in 0.1M phosphate buffer pH 7.0 (PB), washed with 20 drops of PB, incubated with antiserum diluted 1:50 in PB, washed with 20 drops of PB again, and incubated for 4 h with crude leaf extracts of infected *N. benthamiana*. After washing with 40 drops of distilled water and negative staining with UA, the number of particles was counted per 500 μm^2, and means of duplicate grids were calculated.

5 Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed as outlined by Clark and Adams (1977) with the exception that for the tests at Beirut (but not at Braunschweig) the standard extraction buffer was substituted by 0.1M potassium phosphate + 0.1M ethylenediaminetetraacetic acid (EDTA), pH. 7.4. Sap extracts were diluted 1:10 at Beirut and 1:20 or 1:40 at Braunschweig. Substrate reaction times were two hours at Beirut and one hour at Braunschweig. Plates were read at 405 nm in a Pye Unicam SP8-300 spectrophotometer at Beirut and in a Dynatech Microelisa Autoreader MR 580 at Braunschweig. Sources of antisera and homologous antigens used in this study were the following : azuki bean

mosaic virus (AzMV) antiserum provided by N. Iizuka, Japan; blackeye cowpea mosaic virus - necrotic ring spotting isolate BlCMV-NR) - provided by O.W. Barnett, South Carolina; BlCMV-Fla_2 (Florida isolate) and cowpea aphid-borne mosaic virus - Morocco isolate (CAMV-Mor) - provided by D. Gonsalves, New York; BYMV-G (Gladiolus isolate) provided by J.W. Randles, Australia; BCMV-NL 3 and NY 15 provided by G.I. Mink, Washington; BCMV-NL 5 provided by D.Z. Maat, the Netherlands. Virus isolates were also tested by indirect ELISA (Koenig, 1981) against a number of potyvirus antisera by G.I. Mink, Professer, WSU, USA.

Results

1 Host response

The response of the different plant species to the two virus isolates is summarized in Table 1. *Vigna unguiculata* cv. 'California Blackeye No. 5' inoculated with both isolates produced brown necrotic ring lesions followed by a mild systemic mottle in the trifoliate leaves. Isolate 52-82 produced chlorotic local lesions on *C. amaranticolor* followed by latent systemic infection. On *P. vulgaris* cv. 'Monroe', this isolate produced necrotic flecking on the primary leaves followed by a severe mosaic at the growing tip. It also produced vein clearing on systemically infected leaves of *Astragalus sinicus,* leaf puckering and malformation on *P. vulgaris* cvs. 'Black Turtle Soup', 'Bountiful' and 'Sutter Pink'. In addition, isolate 52-82 was latent in *Beta vulgaris* cv. 'Sacchopoly'.

Table 1 Host range of potyvirus isolates 52-82 and 53-82 from *Phaseolus vulgaris* compared to that of azuki beanmosaic (AzMV) and blackeye cowpea mosaic (BlCMV) viruses

Plant species	52-82	53-82	AzMV[a]	BlCMV[a]
Beta vulgaris L. 'Sacchopoly'	La[b]	-	NT	NT
Chenopodium amaranticolor Coste and Reyn. 'Corvallis'	LL, La	La	-	S
Chenopodium quinoa Willd.	-	-	-	LL
Astragalus sinicus L.	S	S	LL, S	NT
Glycine max (L.) Merr. 'Bragg'	-	-	S	LL, S
Phaseolus vulgaris L				
'Bountiful'	S	S	LL, S	LL, S
'Great Northern 1140'	LL, S	-	NT	-
'Black Turtle Soup	LL, S	LL, S		
'Monroe'	LL, S	-		
'Sutter Pink'	S	S		
'Red Mexican 34'	-	-		
'Red Mexican 35'	LL, S	-		
'Redlands Greenleaf C'	LL, S	-		
'Stringless Green Refugee'	LL, S	LL, S		
'Great Northern 31'	LL	-		
'Puregold Wax'	LL, S	-		
Trigonella foenum-graecum L.	-	La	NT	NT
Vicia faba L.	-	-	-	S
Vigna unguiculata (L.) Walp.				
'California Blackeye No. 5'	LL, S	LL, S	LL, S	LL, S
Nicotiana benthamiana Domin.	S	S	NT	S

a Data from Boswell and Gibbs (1983).
b Symptoms abbreviations:
S = Systemic, LL local lesions
La = latent infection, NT = not tested
- = not susceptible.

Unlike isolate 52-82, our isolate 53-82 did not produce local lesions on *C. amaranticolor* and it did not infect *P. vulgaris* cvs. 'Monroe', 'Great Northern 1140', 'Red Mexican 35' and 'Puregold Wax'. In addition, isolate 53-82 was latent in *B. vulgaris* cv. 'Sacchopoly'. Systemic infection was observed in *Trigonella fanum-graecum*. The reactions of the two isolates on *P. vulgaris* differential cultivars as compared to BCMV strains NL 3 and NY 15 are presented in Table 2. None of the IITA-TVu cowpea lines inoculated with the isolate 52-82 was infected (Table 3), whereas when the same lines were inoculated with isolate 53-82 two of them were infected. In one line (IITA-TVu 196) systemic mottle was observed and in the other (IITA-TVu 1582) the infection was latent.

A comparison among the host reactions of isolates 52-82, 53-82, blackeye cowpea mosaic virus and azuki bean mosaic virus is presented in Table 1.

No infection by either isolate was observed on *Gomphrena globosa* L., *Chenopodium quinoa* Wild., *Spinacea oleracea* L. 'Supergreen' and 'Bloom Long Standing', *Cucumis sativus* L. 'Beit

Table 2 Reactions[a] of *Phaseolus vulgaris* cultivars to potyvirus isolates 52-82 and 53-82 and bean common mosaic virus strains NY 15 and NL 3

Phaseolus vulgaris	RCMV[b]		Virus isolates	
cultivars	NY-15	NL-3	52-82	53-82
Sutter Pink	S	S	S	S
Stringless Green Refugee	S	S	S	S
Puregold Wax	T	T	T	T
Redland Greenleaf C	T	T	S	T
Red Mexican 34	S	S	R	R
Monroe	R	R	S	R
Great Northern 31	R	R	R	R
Red Mexican 35	R	R	S	R
Black Turtle Soup	R	S	S	S

a Reactions:
S = Susceptible, T = either mild or latent infection
R = resistant (not infected)
b Data from Drijfhout *et al.*, (1978).

Table 3 Reactions of six cowpea lines mechanically inoculated with blackeye cowpea mosaic virus (BlCMV) (Florida), cowpea aphid-borne mosiac virus (CAMV) (Morocco), and potyvirus isolates 52-82 and 53-82 (Lebanon) as assayed by ELISA[a]

IITA TVu[b] Cowpea line	BlCMV[c]	CAMV[c]	52-82	53-82
196	+	-	-	+
1593	+	-	-	-
1582	+	-	-	+
3273	-	+	-	-
2740	-	+	-	-
3433	-	+	-	-

a + = virus was detected; - = virus was not detected;
b IITA-TVu = International Institute of Tropical Agriculture, Ibadan, Nigeria. Cowpea lines were obtained from L. Bos, The Netherlands.
c Data from Taiwo *et al.* (1982)

Alpha', 'Chicago Pickling', 'Marketer', and 'Tendergreen', *Ocimum basilicum* L., *Cicer arietinum* L. 'IL 482', *Glycine max* (L.) Merr. 'Bragg', *Lens esculenta* Moench. 'Lebanese Local', *Medicago sativa* L. 'Du Puis', *Phaseolus aureus* L., *Phaseolus lunatus* L. 'Lebanese Local', 'Hasbaya', *Trifolium repens* L., *Vicia faba* L. 'Bell Bean' minor, 'Compacta' major, 'Tick Bean' minor, *Vigna angularis* Willd. 'Kyoto Dainagon', *Phlox drummondii* Hook, 'Tall Mixed Color', *Antirrhinum majus* L. 'Tetra Giant Ruffled Mixed Color', *Capsicum annuum* L. 'Pip', *Lycopersicon esculentum* Mill. 'Marglobe', *Nicotiana clevelandii* Gray., *N. glutinosa* L. 'Corvallis', *N. tabacum* L. 'Havana 423', 'White Burley', 'Xanthi', *Petunia hybrida* Vilm. 'F_1 Grandiflora Mixed', *Physalis floridana* Rydv., *Tetragonia expansa* Murr. 'New Zealand'.

2 Electron microscopy

Plants infected with isolates 52-82 or 53-82 contained filamentous particles with normal length values of 812 nm and 797 nm, respectively (400 particles measured). Crude sap preparations contained elements of cylindrical inclusions in the form of scrolls. In ultrathin sections only pinwheel and scrolls could be visualized. Thus, particle morphology and cytopathology suggested the presence of potyviruses.

In ISEM tests (Table 4) particles of both isolates were trapped in high numbers by homologous and heterologous antisera as well as antisera against BlCMV-Fla_2, AzMV and BCMV-NY 15. Lower numbers were trapped by CAMV antiserum, whereas few, if any, particles were trapped by antisera to BYMV and peanut mottle virus. In repeated tests 53-82 showed higher particle concentrations than 52-82 in tissue of *N. benthamiana*.

Table 4 Binding of particles of the two potyvirus isolates from *Phaseolus vulgaris* (52-82 and 53-82) to electron microscope grids coated with protein A and them with antisera to different potyviruses

Antiserum[a]	Particle count virus isolate[b]	
	52-82	53-82
Isolate 52-82	200	1815
Isolate 53-82	180	1815
Azuki bean mosaic virus	248	1580
Blackeye cowpea mosaic virus-Fla_2	245	1235
Bean common mosaic virus NY15	153	1700
Cowpea aphid-borne mosaic virus	39	185
Bean yellow mosaic virus	3	50
Peanut mottle virus	3	30
Normal serum	1	10

a Antiserum dilution was 1:50 and reaction time of virus sample on the EM grids was 4 hours.
b Crude extract from infected *N. benthamiana*.

3 ELISA

Leaf tissues infected with isolates 52-82 and 53-82 and six other potyviruses were tested by direct ELISA using nine different antisera. The A405 values were transformed as percent of the ELISA value of the homologous reaction and are presented in Fig. 1. These data indicated that isolates 52-82 and 53-82 reacted strongly with antisera to each other, and antisera to BlCMV-Fla_2, BlCMV-NR, and AzMV, but less strongly with antisera to BCMV-NY 15 and CAMV (Mor). However, in tests conducted in Braunschweig with a conjugate prepared from the same antiserum to BCMV-NY 15 both isolates yielded much stronger reactions than those shown in Fig.

1. Isolates 52-82 and 53-82 reacted weakly with antisera to CAMV, BYMV, BCMV-NL 3 and NL 5. Tests with six other antigens revealed a strong reaction of BlCMV-NR and BCMV-NY 15 with antisera to 52-82, 53-82, BlCMV-Fla_2, and AzMV. In experiments at Braunschweig (not shown in Fig. 1) strong reactions were also obtained between BlCMV-Fla_2 and antiserum to BCMV-NY 15. Antisera to 52-82 and 53-82 gave weak reactions with CAMV, BYMV, BCMV-NL 3 and NL 5.

The same isolates (52-82 and 53-82) were sent to Dr. Gaylord Mink (Prosser, Washington) and tested against a number of potyviruses antisera using an indirect ELISA procedure. Strongest reaction was obtained with BlCMV antiserum followed by BCMV-NY 15, clover yellow vein virus (ClYVV), pea seed-borne mosaic virus (PSbMV) and BCMV-NL 3 antisera in a decreasing order.

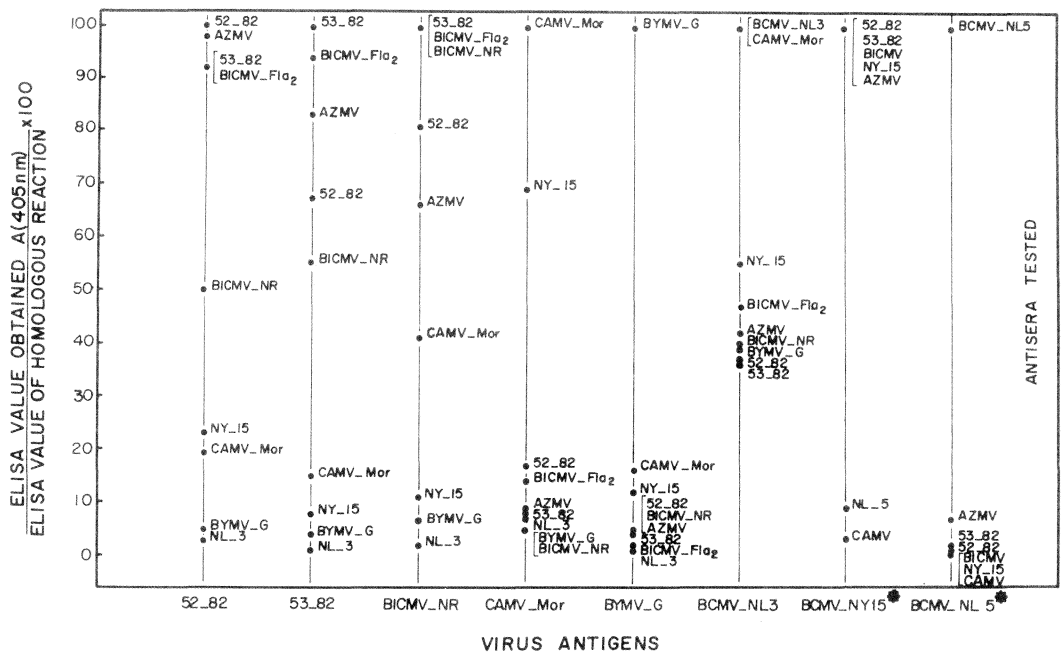

Fig. 1 Relatedness among different potyviruses based on their ELISA (direct) reaction values with homologous and heterologous antisera. *Tests were conducted at Braunschweig using plant sap diluted 1:20 in extraction buffer described by Clark and Adams (1977).

Discussion

Based on host range studies, serology, and immune electron microscopy, the two potyvirus isolates in this study appeared to be closely related to BlCMV, AzMV, and BCMV-NY 15.

Reactions obtained on different plant species indicated that our isolates were different from each other and from BlCMV, BCMV (NY 15 and NL 3) and AzMV (Tables 1 and 2). Many of the host reactions were similar to those reported for BlCMV with the exception that they did not produce lesions on *C. quinoa* and did not infect *Glycine max* or *Vicia faba* systemically (Anderson, 1955 and Lima *et al.*, 1979). Both isolates produced necrotic rings on inoculated leaves of 'California Blackeye No. 5' which is similar to what was reported for a strain of BlCMV from South Carolina (Murphy *et al.*, 1984). Taiwo *et al.*, (1982), using virus reactions to a number of IITA-TVu cowpea lines, were able to clearly differentiate between CAMV and BlCMV. Reactions

obtained with isolate 53-82 on the above same lines indicated that it behaved like BlCMV on two of the three cowpea lines. However, the isolate 52-82 did not infect any of these lines. Likewise, the two isolates showed similarities to as well as differences with BCMV-NY 15 in reactions on *P. vulgaris* cultivars used for differentiating BCMV isolates. Host plant reactions shown in Table 1 also indicated similarities to AzMV. In conclusion, there are as many differences in symptomatology between the two potyviruses under study and among the two isolates and BlCMV, AzMV, or BCMV-NY 15. Therefore, on the basis of host reaction neither of the two potyviruses can be identified specifically as any one of these three previously described potyviruses.

In the ELISA tests, strong reactions were observed between BlCMV-Fla_2, BlCMV-NR, AzMV, and BCMV-NY 15 antisera and 52-82 and 53-82 antigens. Very weak reactions were observed between the above antigens and CAMV, BYMV, and BCMV-NL3 and NL5 antisera. Such results showed a close serological relationship between our isolates and BlCMV, BCMV-NY 15, and AzMV. Using gel immunodiffusion, a positive reaction between two BCMV antigens and BlCMV antiserum has been reported earlier (Lima *et al.*, 1979). The ISEM data confirmed the ELISA results in that they indicated that 52-82 and 53-82 are closely related to AzMV, BlCMV and BCMV-NY 15. They also showed that both isolates are distantly related to CAMV. Consequently, isolates 52-82 and 53-82 seemed to have binding affinities similar to that of AzMV, BlCMV, and BCMV-NY 15.

Differentiation of BlCMV-Fla_2 and CAMV-Mor by direct ELISA obtained in this study was in agreement with a previous report (Taiwo and Gonsalves, 1982). However, in this study BlCMV antiserum gave a strong reaction with BCMV-NY 15 and AzMV antigens and vice versa. Consequently, in field surveys for the detection of BCMV, AzMV, or BlCMV, the strong cross-reactions mentioned above would make it difficult to interpret the ELISA results obtained.

It has been suggested earlier that AzMV is related to or is a strain of CAMV (Bock and Conti, 1974; Boswell and Gibbs, 1983). Data presented here indicate that AzMV is more closely related to BlCMV and BCMV-NY 15 than to CAMV. Work of Taiwo and Gonsalves (1982) and Taiwo *et al.*, (1982) suggests that many legume potyviruses have been erroneously described as CAMV and are better considered as isolates of BlCMV. More comparative work needs to be done to clarify whether or not AzMV, BlCMV and perhaps certain BCMV strains represent different isolates of the same virus.

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Discussion

Rossel, H.W. (IITA): When comparing some isolates of cowpea aphid-borne mosaic virus (CAMV) from Nigeria on Taiwo's set of cowpea differentials, none of the isolates actually fitted one or the other, like in your case in the two bean isolates.

Answer: Most likely the differentials reported by Taiwo *et al.* fitted very well with the black cowpea mosaic virus and cowpea aphid-borne mosaic virus isolates they used for their study, but as you mentioned, they may not be applicable to other isolates.

Honda, Y. (Japan): Have you tried to check the rates of seed transmission of the two virus isolates from *Phaseolus vulgaris?*

Answer: We checked the seed transmission and both potyviruses were seed- transmissible. However the number of seeds we used was low, which did not permit giving a rate for seed transmission.

Tsuchizaki, T. (Japan): Seed transmission tests are very important. Did you test the azuki bean mosic isolates?

Answer: We only worked on the antiserum.