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DETERMINATION OF BROAD BEAN STAIN VIRUS SEROTYPES BY ENZYME-LINKED IMMUNOSORBENT ASSAY

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Summary. – Enzyme-linked immunosorbent assay (ELISA) was used to determine the degree of serological specificity of two serotypes of broad bean stain virus (BBSV) and their relationship to red clover mottle virus (RCMV). Optimal conditions for the differentiation by ELISA of the two viruses and the two BBSV serotypes were established. BBSV isolates from *Vicia sativa* belonged to serotype I, those from pea plants (F1, Kow 60) to serotype II, and isolates from *Lens culinaris* differed from these two serotypes. ELISA revealed no antigenic differences between 22 RCMV isolates which showed the same degree of serological relationship to both BBSV serotypes.

Key words: broad bean stain virus; red clover mottle virus; serotypes; ELISA

Introduction

BBSV (Comovirus group) includes isolates showing a different pathogenicity for certain host plants, especially for certain pea (*Pisum sativum L.)* cultivars. This was the cause why extremely different pathotypes (strains) of the virus were described as distinct viruses: pea green mottle virus (PGMV; Valenta *et al.*, 1969) and pea seed-borne symptomless virus PSbSV (Kowalska and Beczner, 1980). Both these strains differ serologically as does an independent serotype from other BBSV isolates (Musil *et al.*, 1983). This fact along with the finding that both PSbSV and PGMV as well as other BBSV isolates, are seed-borne in pea and other pulse crops (Musil and Kowalska, 1993) led us to determine the conditions of the applicability of ELISA for serotyping of BBSV isolates from Czechoslovakia and Poland.

Materials and Methods

The following virus isolates were used: 10 BBSV isolates of Czechoslovak origin from naturally infected *Vicia sativa L.* (isolates designated VsM, representing serotype I) and *Lens culinaris Med.* (isolates designated LeM); isolate F1 (= PGMV), representing serotype II; isolate Kow 60 (= PSbSV) from Poland; and red clover mottle virus (RCMV) isolates of Czechoslovak origin from red clover (*Trifolium pratense L.*) including the TpM_{36} isolate as representative of the RCMV serotype

widespread in Czechoslovakia (Musil and Gallo, 1984).

All BBSV and RCMV isolates were propagated and purified as described earlier (Musil *et al.* 1983; Musil and Gallo, 1984). Nucleoprotein concentration in the purified suspensions was determined spectrophotometrically. The antigen concentrations used in ELISA were 1000, 500, 250, 125, 62 and 31 ng/ml. Antigen titers in purified suspensions of VsM, F1 and TpM_{36} isolates were determined using serial dilutions of suspensions up to a concentration of 1 ng/ml.

Immunoglobulin (IgG) fractions from hyperimmune rabbit antisera to the VsM, F1 and TpM_{36} isolates (Musil *et al.*, 1983) were prepared and ELISA was carried out as described by Musil and Gallo (1990).

Results and Discussion

Reactions of anti-VsM, anti-F1 and anti-TpM_{36} IgG with homologous and heterologous antigens in ELISA

Samples of serially diluted VsM, F1 and TpM_{36} antigens bound to homologous coating IgG gave a positive reaction with optimally diluted homologous conjugates also at a nucleoprotein concentration of only 0.25 ng/ml. The absorbancy values obtained with serial antigen dilutions were proportional to the antigen concentration within the range of nucleoprotein concentrations from 100 to 1 ng/ml.

The titer of labelled antibody in anti-VsM, anti-F1 and anti-TpM_{36} IgG against homologous antigens, with the use of homologous coating IgG, corresponded to dilutions of 1:256 000 (anti-VsM and anti-F1 conjugates) and 1:128 000 (anti-TpM_{36} conjugate). The absorbancy values obtained in reactions of gradually diluted conjugates with homologous antigens were proportional to the dilution, starting from the dilution of 1:4000.

Dilution of the coating IgG from 1:500 to 1:4000 had no marked effect on the intensity of homologous or heterologous reactions. To determine the antigenic relationships between VsM and F1 isolates and anti-VsM and anti-F1 IgG on the one hand, and their relationship to RCMV (TpM_{36}) on the other hand, we used coating IgG diluted 1:1000, conjugate dilutions from 1:1000 to 1:4000, and antigen concentrations 100 and 50 ng/ml. The results of homologous and heterologous reactions are summarized in Table 1.

It is evident that the relationship between VsM and F1 antigens or that between their corresponding antibodies in anti-VsM and anti-F1 IgG is closer than between VsM or F1 antigen and TpM_{36} antigen. The antibody in anti-TpM_{36} IgG reacted substantially less intensively with both VsM and F1 antigens than with the homologous antigen (absorbancy values 0.24–0.45). Similarly, the intensity of reaction of the antibody in anti-VsM or anti-F1 IgG with TpM_{36} antigen was also comparatively low (absorbancies 0.24–0.44 and 0.35–0.51, respectively). By contrast, the absorbancy values determined in reactions of anti-VsM IgG with F1 antigen or of anti-F1 IgG with VsM antigen were substantially higher than in the reaction with TpM_{36} antigen. E. g., in the reaction of anti-VsM conjugate with F1 antigen bound to anti-VsM IgG the absorbancy values were only 2–4 times lower than those in the homologous reaction. Similarly, in the

reaction of anti-F1 conjugate with VsM antigen bound to anti-F1 IgG the absorbancy values were by 1/3 lower than those in the homologous reaction. But in the reaction of either anti-VsM or anti-F1 conjugate with TpM_{36} antigen bound to anti-VsM or anti-F1 coating IgG the absorbancy values were 6–10 times lower than those in the homologous reaction and no definite positive reactions were obtained with conjugates diluted 1:4000.

Table 1. Reaction of anti-VsM, anti-F1 and anti-TpM_{36} conjugates with homologous and heterologous antigens in ELISA

ELISA coat-antigen-conjugate	A_{410}			
	Conjugate 1:1000		Conjugate 1:4000	
	Antigen concentration		Antigen concentration	
	I	II	I	II
VsM-VsM-VsM	3.00	2.55	1.84	1.02
VsM-F1-VsM	1.49	0.81	0.55	0.27
VsM-TpM_{36}-VsM	0.24	0.24	0.12	0.10
F1-VsM-VsM	3.58	3.06	2.06	1.43
TpM_{36}-VsM-VsM	2.14	1.68	1.08	0.78
F1-F1-VsM	2.18	1.09	0.72	0.37
TpM_{36}-TpM_{36}-VsM	0.44	0.27	0.15	0.14
TpM_{36}-F1-VsM	0.82	0.55	0.29	0.18
F1-TpM_{36}-VsM	0.40	0.37	0.15	0.13
F1-F1-F1	3.12	2.16	1.51	0.92
F1-VsM-F1	2.44	1.62	0.99	0.64
F1-TpM_{36}-F1	0.51	0.34	0.17	0.14
VsM-F1-F1	3.11	2.12	1.22	0.78
TpM_{36}-F1-F1	1.56	1.30	0.63	0.36
VsM-VsM-F1	2.15	1.43	0.90	0.53
TpM_{36}-TpM_{36}-F1	0.50	0.25	0.19	0.15
TpM_{36}-VsM-F1	0.99	0.99	0.53	0.41
VsM-TpM_{36}-F1	0.35	0.19	0.14	0.12
TpM_{36}-TpM_{36}-TpM_{36}	3.18	2.55	1.52	0.90
TpM_{36}-VsM-TpM_{36}	0.23	0.19	0.17	0.17
TpM_{36}-F1-TpM_{36}	0.22	0.20	0.18	0.15
VsM-TpM_{36}-TpM_{36}	1.53	0.91	0.35	0.28
F1-TpM_{36}-TpM_{36}	1.51	0.90	0.32	0.22
VsM-VsM-TpM_{36}	0.39	0.34	0.17	0.13
F1-F1-TpM_{36}	0.44	0.43	0.18	0.14
F1-VsM-TpM_{36}	0.45	0.34	0.17	0.13
VsM-F1-TpM_{36}	0.26	0.22	0.15	0.12

IgG used for coating was diluted 1:1000.
I - 100 ng/ml, II - 50 ng/ml, A_{410} - absorbancy at 410 nm.

The outcome of reactions of individual conjugates with homologous and heterologous antigens bound in further combinations to coating IgG suggest that the results depended on the representation of individual antigenic determinants on the surface of the virions of the BBSV and RCMV isolates tested and on the representation of corresponding antibody groups in the IgG used. In general, the results of the present comparative tests confirmed our previous finding of antigenic differences between RCMV and BBSV (Gallo and Musil, 1988). At the same time the present experiments showed that even distinct BBSV serotypes showed almost the same serological relationship to RCMV. The serological differences between BBSV serotypes are due to other antigenic determinants and their corresponding antibodies and thus differ from the serological differences between representatives of the Comovirus group, as suggested previously (Musil *et al.*, 1983).

Differences in homologous and heterologous reactions of anti-VsM and anti-F1 IgG with VsM and F1 antigens, representing two distinct BBSV serotypes (serotype I – VsM isolate, serotype II – F1 isolate) suggested the possibility of using ELISA for serotyping of BBSV isolates.

Serotyping of BBSV and RCMV isolates

When using ELISA in practice for the detection of BBSV in naturally infected plants, two types of reaction can be anticipated in the case of two distinct serotypes. On of the possible reactions is, in general, identical with homologous reaction, namely when the detected antigen belongs to the same serotype as the antibody used in the test. The second type of reaction is a kind of heterologous reaction, namely when the detected antigen belongs to another serotype than the antibody used in the test. We tested as to how far these two types of reaction would become manifested in serotyping of the available BBSV isolates. All ten BBSV isolates tested showed the same degree of antigenic diversity from RCMV. BBSV isolates obtained in different years from naturally infected *Vicia sativa* plants in the localities Horná Streda and Šumperk belonged to serotype I, because these isolates reacted with anti-VsM IgG with the same intensity as in the homologous reaction. With anti-F1 IgG these isolates gave a less intensive reaction than that with homologous F1 antigen. Isolate Kow 60 (= PSbSV) was found to belong to serotype II; like F1, it reacted with anti-VsM IgG less intensively than with anti-F1 IgG. BBSV isolates obtained from naturally infected lentil plants (LeM/1, LeM/18 and LeM/20) reacted with either anti-VsM IgG or anti-F1 IgG less intensively than with VsM or F1 antigen in the homologous reaction. These results suggest that lentil isolates could represent a further independent serotype of BBSV (Table 2).

In parallel with BBSV isolates we also tested 22 RCMV isolates against anti-VsM, anti-F1 and anti-TpM_{36} IgG. All RCMV isolates reacted with anti-TpM_{36} IgG with an intensity corresponding to that of the homologous reaction. By contrast, all RCMV isolates reacted with anti-VsM or anti-F1 IgG with an

Table 2. Reaction of BBSV and RCMV isolates with anti-VsM, anti-F1 and anti-TpM_{36} IgG

Isolates		A_{410} Antigen concentration anti-VsM IgG		anti-F1 IgG		anti-TpM_{36} IgG	
		I	II	I	II	I	II
BBSV:	VsM	1.73	1.75	1.47	1.71	0.12	0.12
	VsM/15	1.66	1.53	1.28	1.28	0.11	0.10
	VsM/24	1.47	1.57	1.38	1.22	0.10	0.10
	VsM-31	1.74	1.67	1.60	1.53	0.11	0.10
	VsM/32	1.75	1.62	1.52	1.50	0.09	0.09
	VsM/Š	1.53	1.53	1.49	1.34	0.11	0.10
(PGMV)	F1	0.70	0.45	1.89	1.88	0.11	0.10
(PSbSV)	Kow 60	0.51	0.42	1.68	1.44	0.09	0.09
	LeM/1	0.54	0.44	0.61	0.72	0.11	0.10
	LeM/18	0.62	0.37	0.86	0.67	0.10	0.10
	LeM/20	0.74	0.64	0.95	0.78	0.11	0.10
RCMV:	TpM_{36}	0.12	0.10	0.17	0.14	1.52	1.32
	TpM_{25}	0.12	0.11	0.16	0.15	1.47	1.27
	No. 1 to 25	0.12	0.11	0.18	0.14	1.50	1.18

IgG used for coating was diluted 1:1000.
Conjugate was diluted 1:4000.
I - 1000 ng/ml, II - 100 ng/ml, A_{410} - absorbancy at 410 nm.

intensity corresponding to that of heterologous reactions VsM-TpM_{36}-VsM or F1-TpM_{36}-F1.

The present results confirmed that DAS ELISA can be used for serotyping of BBSV isolates. These results also showed that in using ELISA for BBSV detection in naturally infected plants it is necessary to take into account the different reactivity of BBSV serotypes.

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