Sensitivity of Dot-ELISA on Nitrocellulose Membranes in Comparison with ELISA on Polystryrene Plates for the Detection of Four Plant Viruses

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ABSTRACT

Sensitivity of dot-immunobinding ELISA on nitrocellulose membrane (Dot-ELISA) was compared with double-antibody sandwich ELISA (DAS-ELISA) on polystyrene plates for the detection of bean yellow mosaic virus (BYMV), broad bean stain virus (WMV-2). Dot-ELISA was 2 and 10 times more sensitive than DAS-ELISA for the detection of BBSV and WMV-2, respectively, whereas DAS-ELISA was more sensitive than Dot-ELISA for the detection of BYMV. Both techniques were equally sensitive for the detection of BYDV. Using one day instead of the two-day procedure, the four viruses were still detectable and the relative sensitivity of both techniques remained the same.

INTRODUCTION

Double-antibody sandwich ELISA (DAS-ELISA) is widely used for plant virus detection using polystyrene microtiter plates as the solid support (Clark and Adams, 1977). The use of nitrocellulose membranes (NCM) as the solid support, where antigens to be tested are adsorbed as dots (Dot-ELISA), was recently reported (Banttari and Goodwin, 1985; Batteiger *et al.*, 1982; Parent *et al.*, 1985; Rybicki and Von Wechmar, 1982). For a number of plant viruses investigated so far, Dot-ELISA proved to be more sensitive than DAS-ELISA (Banttari and Goodwin), 1985; Parent *et al.*, 1985).

In this study we evaluated the sensitivity of both DAS- and Dot-ELISA in detecting BBSV, BYMV, BYDV and WMV-2 in infected tissue.

MATERIALS AND METHODS

Virus Cultures and Antisera

The BBSV culture (LV 140-85), and the WMV-2 culture (1-84) used were from Lebanon. Two isolates of BYMV were used, one from Australia (BYMV-G) provided by Dr.J.W.Randles, Waite Agriculture Research Institute, Adelaide, and the other from Syria (SV 21-85). The BYDV culture was from Tunis (TB7-85). The BYMV, BBSV and BYDV isolates from Syria, Lebanon, and Tunis, respectively, were collected earlier during the spring of 1985 and were identified in our laboratory.

The BBSV antiserum was provided by Dr.A.A.Brunt, Glasshouse Crops Research Institute, Littlehampton, England. The WMV-2 antiserum was provided by J.A.Dodd, University of California at Riverside, USA. BYMV-G antiserum was provided by J.W.Randles and BYMV-B25 antiserum was provided by D.Z.Maat, IPO, Wageningen, The Netherlands. BYDV antiserum was from Inotech Diagnostik, Basel, Switzerland.

ELISA Procedure

The DAS-ELISA procedure followed was that of Clark and Adams (1977). The Dot-ELISA procedure, including the use of a plastic template for placing the samples on NCM, was that of Banttari and Goodwin (1985). The templates used were 10mm thick and the holes were 5mm in diamter. Samples of 200 ul of sap were placed in each hole. Three layers of Whatman no. 1 filter paper were placed under the NCM. Duplicate holes were used for each sample tested. The NCM used was from BIO-RAD Laboratories, Richmond, California (No. 162-0116).

In the one day shortened procedure for both DAS- and Dot-ELISA, coating of gamma globulins was for 1 hr, sample addition for 3 hr, enzyme conjugate addition for onehr and substrate incubation for 30 min.In case of Dot-ELISA, the blocking agent (Banttari and Goodwin, 1985) was added for 30 min after coating with gamma globulins. Gamma globulin concentration of 1ug/ml and conjugate dilution of 1/1000 were used.

RESULTS

When the sample extraction buffer TBS-T80 (Tris- HCL buffer, pH 7.4, containing 0.9% NaCl and 0.05 Tween 80) plus 0.01M sodium diethyldithiocarbamate (Na DIECA) reported by Banttari and Goodwin (1985) for use in Dot-ELISA was compared with 0.2M phosphate buffer, results obtained indicated that both buffers were equally suitable for the detection of BBSV in Dot- and DAS-ELISA. In addition, when TBS-T80 + 0.01M Na DIECA was compared with 0.1M phosphate buffer, pH 7.4, containing 0.1M EDTA for sample extraction of WMV-2-infected tissue, both buffers produced comparable results for the detection of WMV-2 by Dot-ELISA (Table 1).

The suitability of the 0.2M phosphate buffer, pH 6.0 and 0.1M phosphate buffer, pH 7.4, for the detection of BBSV and WMV-2, respectively, in Dot-

ELISA was tested because these two buffers proved to be efficient for the detection of these two viruses by DAS-ELISA in polystyrene plates (unpublished). Based on these results 0.1M phosphate buffer, pH 7.4, containing 0.1M EDTA was used in the subsequent tests for WMV-2 detection and 0.2M phosphate, pH 6.0, for the detection of BBSV, BYMV and BYDV in both DAS- and Dot-ELISA.

BYMV was detectable at 1:2000 sap dilution with DAS-ELISA and only at 1:100 dilution with Dot-ELISA. WMV-2 was detected at 1:1000 dilution by Dot-ELLISA and only at 1:100 dilution with DAS-ELISA. Similarly, BBSV was detected at 1:8000 dilution by Dot-ELISA and only at 1:4000 dilution by DAS-ELISA. BYDV was equally detectable by both techniques (Table 2).

In case of healthy broad bean tissue, dark greem dots were always obtained on the NCM. Addition of 5% Na_2SO_3 as an antioxidant to the extraction buffer produced spots which are lighter in color, but the sensibility of the test was not improved.

Even though BYDV was equally detected by DAS-and Dot-ELISA, visibility of the reaction was better with Dot- than with DAS-ELISA. Virus concentration in BYDV-infected sample influenced the extent of the dilutions (Fig. 1). Sample A (Fig. 1, A) when diluted 100 times was still recognized as positive, whereas in sample B (Fig. 1, B), BYDV could not be detected beyond 10× sample dilution. A 405 values of 0.12-0.18 were not easily determined visually in DAS-ELISA, whereas the dots produced by the same samples on nitrocellulose membranes were much easier to distinguish visbility as compared to the healthy controls (Fig. 1, B). It should be mentioned here, however, that resolution of the actual stained membrane is better than in the black and white picture of Fig. 1.

When the procedure was reduced to one working day, the sensitivity of both DAS-and Dot-ELISA was reduced. In Dot-ELISA, BYMV, WMV-2, BBSV

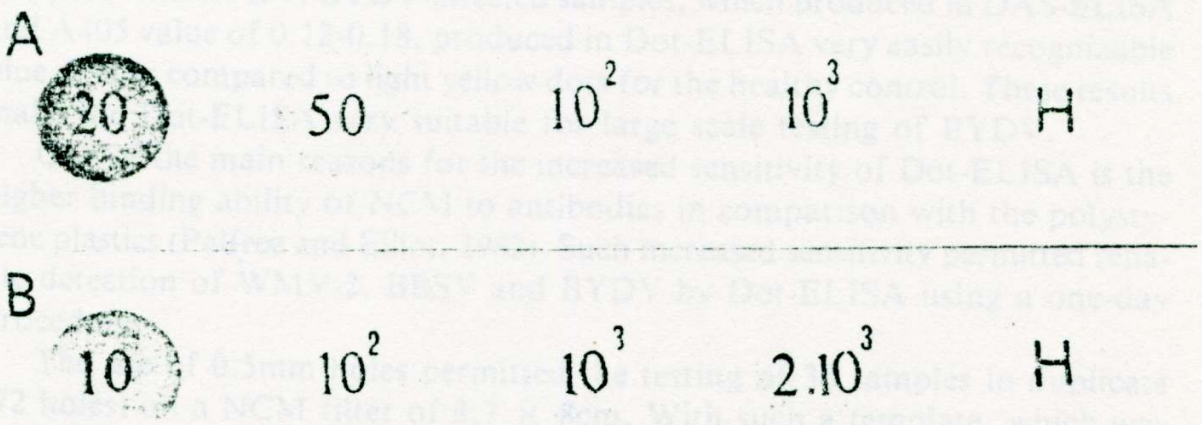

Fig. 1. A nitrocellulose membrane from Dot-ELISA for two samples (A and B) of barley yellow dwarf virus (BYDV)-infected barley leaves. The numbers are reciprocal dilutions and "H" is for healthy barley. In the first sample (A) the corresponding A405 value in DAS-ELISA for the BYDV-infected sample (20x dilution) was 0.88 and the corresponding A405 value for the second sample (B) (10x dilution) was 0.12.

and BYDV were detectable in sap dilutions of 1:20 (weakly), 1:20, 1:16000 and 1:10, respectively. In DAS-ELISA, the above four viruses were detected at 1:20 (weakly), 1:20 (weakly), 1:2000 and 1:10, respectively (Table 3).

DISCUSSION

The sample extraction buffer 0.2M phosphate, pH 6.0, was as efficient as the TBS-T80 buffer, pH 7.4, plus 0.01M Na DIECA reported by Banttari and Goodwin (1985) for use in Dot-ELISA. In our laboratory, this low pH extraction buffer proved to be efficient for the detection of BYMV, BBSV and BYDV in DAS-ELISA. The high efficiency of BYDV detection in DAS-ELISA using 0.1M phosphate buffer, pH 6.0, has been reported previously (Clement *et al.*, 1984).

Healthy broad bean tissue tended to produce green dots on the NCM as compared to light yellow dots produced by squash or barely tissue. The addition of 5% Na_2SO_3 to the extraction buffer helped in producing dots which were lighter in color, but it did not improve the sensitivity of the test, which is in agreement with a previous finding (Banttari and Goodwin, 1985).

Dot-ELISA (2-day procedure) was more sensitive than DAS-ELISA for the detection of BBSV and WMV-2 and equally sensitive for the detection of BYDV. However, DAS-ELISA was more sensitive than Dot-ELISA for the detection of BYMV. The same results were obtained when BYMV-G or BYMV (SV21-85) infected tissues were tested by homologous antisera. When the above tests were repeated, the pattern of sensitivity mentioned above was unchanged. It is not clear why in the case of BYMV, Dot-ELISA was less sensitive than DAS-ELISA.

The best contrast between healthy and infected tissue in Dot-ELISA was obtained with BYDV. BYDV-infected samples, which produced in DAS-ELISA and A405 value of 0.12-0.18, produced in Dot-ELISA very easily recognizable blue dots as compared to light yellow dots for the healthy control. These results make the Dot-ELISA very suitable for large scale testing of BYDV.

One of the main reasons for the increased sensitivity of Dot-ELISA is the higher binding ability of NCM to antibodies in comparison with the polystyrene plastics (Palfree and Elliot, 1982). Such increased sensitivity permitted reliable detection of WMV-2, BBSV and BYDV by Dot-ELISA using a one-day procedure.

The use of 0.5mm holes permitted the testing of 36 samples in duplicate (72 holes) on a NCM filter of 8.7 × 8cm. With such a template, which was locally made, the cost of the test per sample was low. The NCM filters are cheaper than the polystyrene microtiter plates, and using them would reduce the cost per sample, even if the plates are washed and reused. In addition, the use of the NCM filter as one unit makes washing and recollection of both gamma globulins and enzyme conjugate easier.

The visual assessment of reactions in Dot-ELISA was, in general, better than DAS-ELISA especially for low virus oncentrations. Thus, Dot-ELISA would be very useful for laboratories where spectrophometers or ELISA readers are not availabale.

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TABLE 1

Detectability of watermelon mosaic virus 2 (WMV-2) in different leaf sap dilutions extracted by two buffers using double-antibody sandwich ensyme-linked immunosorbent assay (DAS-ELISA) and Dot-ELISA

	TBS-T80[b] + 0.01M Na DIECA		0.01M PO_4 + 0.1M EDTA, pH7.4	
Sap dilution	DAS-ELISA	Dot-ELISA	DAS-ELISA	Dot-ELISA
1:20	0.675[d] (+)	+[e]	0.624 (+)	+
1:100	0.354 (+)	+	0.394 (+)	+
1:1000	0.104 (±)	±	0.078 (−)	+
1:4000	0.053 (−)	±	0.029 (−)	±
1:16000	0.041 (−)	−	0.011 (−)	−
Healthy	0.009 (−)	−	0.001 (−)	−

[a] Mircroplates for DAS-ELISA and nitrocellulose membranes for Dot-ELISA were coated with lug/ml gamma globulin. A 1:1000 enzyme-conjugate dilution was used and a 1h incubation for the substrate.
[b] TBS-T80 = 0.01M Tris-HCl buffer, pH 7.4 + 0.9% NaCl + 0.05% Tween 80.
[c] Dilutions were parts sap from infected leaves: parts sap from healthy leaves (v/v).
[d] Values are the absorbancy at 405mm.
[e] ± = positive, − = negative, and + = slightly positive as compared to the healthy control.

TABLE 2

Detection of bean yellow mosaic virus (BYMV), watermelon mosaic virus 2 (WMV-2), broad bean stain virus (BBSV), and barley yellow dwarf virus (BYDV) bu double-antibody sandwich ensyme-linked immunosorbent assay (DAS-ELISA) on polystyrene plates and on nitrocellulose membrane filters (Dot-ELISA)[a] using a two-day procedure

	BYMV		WMV-2		BBSV		BYDV	
Sap dilution[b]	DAS-ELISA[c]	Dot-ELISA	DAS-ELISA	Dot-ELISA	DAS-ELISA	Dot-ELISA	DAS-ELISA	Dot-ELISA
1:20	0.47(+)[d]	+[d]	0.62(+)	+	1.22(+)	+	0.88(+)	+
1:100	0.38(+)	+	0.39(+)	+	*	*	0.27(+)	+
1:1000	0.23(+)	±	0.07(−)	+	0.67(+)	+	0.10(−)	−
1:2000	0.17(+)	−	0.03(−)	±	0.42(+)	+	*	*
1:4000	0.14(±)	−	0.03(−)	−	0.23(+)	+	*	*
1:8000	0.14(±)	−	0.01(−)	−	0.10(−)	+	*	*
1:16000	0.13(±)	−	0.01(−)	−	0.07(−)	±	*	*
1:32000	0.10(−)	−	*[e]	*	0.05(−)	−	*	*
Healthy	0.07(−)	−	0.00(−)	−	0.02(−)	−	0.07(−)	−

[a] Polystyrene plates and nitrocellulose membranes were coated with lug/ml gamma globulin, enzyme conjugate dilution used was 1/1000 and substrate incubation time was one hour.
[b] Dilutions were parts sap from infected leaves: parts sap from healthy leaves (v/v).
[c] Values are absorbancy at 405mm.
[d] Visual observations: + = positive, − = negative, + = very weakly positive as compared to the healthy control.
[e] Not tested.

TABLE 3

Detection of bean yellow mosaic (BYMV), watermelon mosaic virus 2 (WMV-2), broad bean stain virus (BBSV) and barley yellow dwarf virus (BYDV) by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA)[a] on polystyrene plates and on nitrocellulose membrane filters (Dot-ELISA)[a] using a one day procedure

	BYMV		WMV-2		BBSV		BYDV	
Sap dilution[b]	DAS-ELISA[c]	Dot-ELISA	DAS-ELISA	Dot-ELISA	DAS-ELISA	Dot-ELISA	DAS-ELISA	Dot-ELISA
1:10	*	+	*	+	*	+	0.36(+)	+
1:20	0.15(+)[d]	+[d]	0.18(+)	+	0.56(+)	+	s 1,5;0.08(−)	−
1:100	0.11(±)		0.14(+)	+	0.31(+)	+	0.05(−)	−
1:1000	0.04(−)		0.06(−)	−	0.26(+)	+	0.02(−)	−
1:2000	0.03(−)		0.09(−)	−	0.17(+)	+	*	*
1:4000	*[e]	+	*	+	0.15(+)	+	*	*
1:8000	*	+	*	*	0.10(−)	+	*	*
1:16000	*	+	*	*	0.05(−)	+	*	*
Healthy	0.03		0.05		0.01	−	0.02	−

[a] Coating with gamma globulins (1ug/ml) was for 1 hour at 37° C, sample addition was for 3 hours at room temperature, enzyme conjugate (1:1000) addition was for 1 hour at 37° C and substrate incubation time was for 30 min.

b, c, d, e Same as in Table 2.