Detection of plum pox virus in leaves and aphids by SIBA and DAS-ELISA assays

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Abstract

The slot-immunobinding assay (SIBA) was adapted for detection of plum pox virus (PPV) and compared with DAS-ELISA. SIBA was easy to perform and as sensitive as DAS-ELISA in detection of various PPV isolates in herbaceous and woody plants, but not in aphids (*Myzus persicae*).

Introduction

Plum pox virus (PPV) is economically the most important viral disease of stone fruits (for review see Nemeth 1986). PPV is a nonpersistent aphid-transmitted potyvirus with filamentous virions 764 × 20 nm. The nucleic acid of PPV is a simple infective molecule with a M_r of about 3.5×10^6. In the protein capsid three polypeptides were separated with M_r values of 27 000, 29 000 and 43 500 (Kerlan and Dunez 1976). Sutič *et al.* (1971) ranged the virus into 3 strains according to the symptoms caused on *Chenopodium foetidum*: yellow, intermediate and necrotic strains.

Serological methods have been developed for PPV diagnosis, including the latex test, radial gel - diffusion test (Casper 1975) and ELISA test (Clark and Adams 1977). DAS-ELISA, the most widely used method for PPV detection is relatively easy to perform and highly sensitive, but does require some special equipment. A simple and rapid serological method, dot(slot)-immunobinding assay (DIBA, SIBA) has been developed for the detection of several plant viruses (Powell 1987). The entire test can be performed within 6 h with minimal laboratory equipment, using polyclonal antibodies and Protein A labelled with alkaline phosphatase .

The objective of this research was to adapt SIBA for diagnosis of PPV using polyclonal antibodies and to compare the sensitivity of SIBA with DAS-ELISA.

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Material and methods

Virus isolates: Isolates NAT, B2 and NL (intermediate strains) and isolates GS, B3, BS and SCH (yellow strains) of PPV were used for the experiments. Isolate NL originated from the Netherlands, the others originate from BBA Braunschweig. All isolates except SCH were kept on systemically infected *Nicotiana clevelandii*. Isolate SCH was kept on *Prunus domestica* seedlings. All isolates were transmitted to *Chenopodium foetidum* plants by means of mechanical inoculation.

Aphids: Green peach aphids (*Myzus persicae*) were bred on rape (*Brassica napus*) in the greenhouse. Aphids were starved for 2 h in Petri dishes before 1 h acquisition feeding.

ELISA: The ELISA method (Clark and Adams 1977) was used to detect virus in leaves and aphids. Microtitre plates (*Dynatech*) were coated with anti-PPV polyclonal IgG, prepared in BBA Braunschweig, at a dilution of 1:500 with carbonate buffer at pH 9.6. Plates were incubated for 4 h at 37 °C. Leaf tissue was extracted at 1:30 (m/v) in phosphate-buffered saline (PBS) plus 0.05 % Tween 20 (pH 7.4) containing 2 % polyvinylpyrrolidone 40 (PVP). After 1 h of acquisition feeding, aphids were ground with 0.3 cm^3 of extraction buffer in a glass micro-homogeniser, and 0.2 cm^3 of leaf and aphid samples added to the wells and left overnight at 4 °C.

Alkaline phosphatase enzyme conjugate was diluted at 1:500 with PBS + Tween + PVP and added to the wells for 4 h at 37 °C. After addition of *p*-nitrophenyl phosphate substrate at 1 mg cm^{-3} in 10 % diethanolamine buffer at pH 9.8, the plates were left at room temperature in dark for 1 h. Absorbance values at 405 nm were recorded using a *Titertek Multiskan MCC/340 Reader*. The plates were washed with PBS + Tween buffer after each step. All buffers contained 0.02 % sodium azide as preservative.

SIBA: Leaf tissues were extracted 1:20 (m/v) in TBS (20 mM Tris base, 150 mM NaCl, pH 7.5) + 50 mM DIECA. 0.8 cm^3 of the extract was pipetted into a 1.5 cm^3 microcentrifuge tube, 0.4 cm^3 chloroform added, capped, vortexed to mix and centrifuged at 12 000 *g* for 2 min. Then 0.2 cm^3 of the aqueous layer was added to 0.8 cm^3 of TBS + DIECA and vortexed to mix. After 1 h of acquisition feeding aphids were homogenized with 0.3 cm^3 TBS-DIECA buffer and centrifuged at 12 000 *g* for 5 min. Samples (0.1 cm^3) were applied to wet nitrocellulose membrane (*Schleicher & Schuell* 0.45 μm) using a manifold (*Manifold II, Schleicher & Schuell*) and then air-dried.

Dry membrane was floated on 50 cm^3 of blocking solution (TBS + 1 % non-fat dried milk + 0.5 % BSA) until rehydrated, and then gently shaked for 1 h at room temperature. After one rinsing in washing buffer (TBS + Tween 20) for 10 min membrane was incubated at room temperature in primary IgG, diluted 1:500 in TBS + 2 % PVP + 0.2 % BSA 1.5 h. Then the membrane was rinsed three times for *ca.* 10 min in washing buffer, and incubated 1.5 h at room temperature in protein A alkaline

phosphatase labelled conjugate diluted 1:5 000 in TBS + 2 % PVP + 0. 2% BSA + 0.2 % $MgCl_2$. After rinsing four times as above the membrane was placed in a substrate colour development solution (15 cm^3 H_2O + 6 mg naphtol AS-MX phosphate + 90 mg fast-red salt in 15 cm^3 0.2 M Tris-HCl pH 8.0 + 2 mM $MgCl_2$), and incubated until the development of a positive reaction. The colour reaction was stopped by transferring the membrane to distilled water.

Results

Leaves of *N. clevelandii* plants systemically infected with intermediate strains of PPV had 6 - 11 d after inoculation massive spots of a light green colour, while *Ch. foetidum* plants infected with intermediate strains and yellow strains showed ochre-yellow lesions and light not very clear spotting, respectively on the inoculated leaves 5 - 9 d after inoculation. On inoculated leaves of *Ch. foetidum* ochre-yellow spots were visible 7 - 10 d after inoculation.

Detection of PPV in plant tissue by ELISA: Polyclonal antibodies detected PPV in all infected *N. clevelandii, Ch. foetidum* and *P. domestica* plants (Tables 1 and 2). *N. clevelandii* plants systemically infected with intermediate strains of PPV (A_{405} = 0.5. 8 - 0.672) showed the highest absorbance. *N. clevelandii* systemically infected with yellow strains of PPV gave values from 0.226 to 0.595. Absorbance values of nonsystemically infected *Ch. foetidum* plants ranged from 0.213 to 0.373. Absorbance values of a healthy *N. clevelandii* extracts were 0.046 - 0.068. An absorbance higher than negative mean + 3s was considered to be a positive threshold (Sutula *et al.* 1986). The results achieved show that by means of DAS-ELISA using polyclonal antibodies against PPV it is possible to detect PPV reliably at a concentration of about 6 - 8 ng cm^{-3} of plant extract, diluted 1 : 30 (m/v).

Table 1. Comparison of relative sensitivity of DAS-ELISA (average A_{405} from two replicates) and Slot-immunobinding assay (SIBA, visual observation) using polyclonal antibodies against PPV.

PPV isolates	Plants					
	N. clevelandii		*Ch. foetidum*		*P. domestica*	
	ELISA	SIBA	ELISA	SIBA	ELISA	SIBA
NL	0.626/+	+	0.359/+	+		
NAT	0.542/+	+	0.373/+	+		
B3	0.470/+	+	0.292/+	+		
GS	0.278/+	+	0.213/+	-		
SCH					0.254/+	+
Control	0.078	-	0.067	-	0.072	-

+ positive, - negative.

Detection of PPV in plant tissue by SIBA: Typical results obtained with SIBA are illustrated in Fig 1. Polyclonal antibodies consistently gave a positive reaction with

extracts of *N. clevelandii* systemically infected with both strains of PPV within the first 1 min of reaction with the substrate solution, but the positive reaction of intermediate strains was stronger than that of yellow strains. All infected *Ch. foetidum* and *P. domestica* gave a slight positive reaction with polyclonal antibodies. Cross absorption of polyclonal antibodies with healthy *N. clevelandii* plant extracts caused a slight pink nonspecific reaction. The results achieved show that by using SIBA it is possible to detect PPV reliably at a concentration of about 8 ng cm^3 of 1:80 (m/v) diluted plant extract.

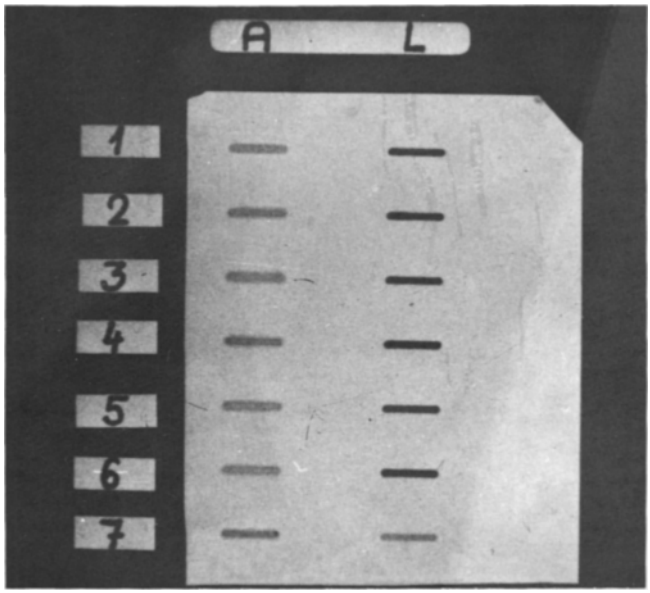

Fig. 1. Slot-immunobinding assay to detect various PPV isolates in *N. clevelandii* leaves (L) and green peach aphid (A) extracts. Row 1 - NAT isolate; row 2 - B2 isolate; row 3 - GS isolate; row 4 - B3 isolate; row 5 - BS isolate; row 6 - NL isolate; row 7 - healthy control.

Table 2. Comparison of relative sensitivy of DAS-ELISA (average A_{405} from two replicates) and Slot-immunobinding assay (SIBA, visual observation) to detect virus particles in plants and aphids using polyclonal antibodies against PPV.

PVV isolate	*N. clevelandii*		Aphids	
	ELISA	SIBA	ELISA	SIBA
NAT	0.561	+	0.039	-
B2	0.672	+	0.042	-
GS	0.226	+	0.057	-
B3	0.595	+	0.049	-
BS	0.385	+	0.053	-
NL	0.538	+	0.038	-
Control	0.053	-	0.042	-

+ positive, - negative.

Detection of PPV in green peach aphid tissue: Aphids were ground in a glass microhomogeniser in batches of 30, 20, 10, 1 (Table 3) and 15 (Table 2, Fig. 1) individuals after 1-h acquisition feeding on systemically infected leaves of *N. clevelandii*. PPV was not detected by polyclonal IgGs in single aphids or in batches of 10, 15, 20 or 30 individuals. In ELISA very low absorbance values (0.033-0.058) were obtained and in SIBA all samples gave a slight pink nonspecific negative reaction.

Table 3. ELISA (average A_{450} from two replicates) detection test for PPV in green peach aphids after 1-h of acquisition feeding on the source of infection.

Aphid batches	PPV isolates NAT	GS	control
1	0.038/-	0.033/-	0.039
10	0.039/-	0.052/-	0.042
20	0.047/-	0.057/-	0.046
30	0.044/-	0.058/-	0.047

- negative

Discussion

Neither method (ELISA, SIBA) detected any PPV isolate in green peach aphids after 1-h acquisition feeding, perhaps reflecting a low titre of the virus in those aphids. Thus the serological methods used are not sensitive enough to detect the small amount of virus carried by individual insects. Virus acquisition must generally therefore be estimated by inoculation and subsequent symptom expression in plants (Powell *et al.* 1992), although Kotúč (unpublished) detected PPV in batches of 14 and 20 *M. persicae* individuals collected from *P. domestica* trees infected with the yellow strain of PPV, using polyclonal antibodies and DAS-ELISA.

Banttari and Goodwin (1985) reported that DOT-ELISA (ELISA on nitrocellulose membranes, similar to our SIBA) was more sensitive than direct DAS-ELISA for the detection of PVX, PVY and PVS in leaf sap of greenhouse and field grown potato plants. They spotted 400 µl of sap plus additives, using plastic templates to confine the plant sap in wells on the NCM. Berger *et al.* (1985) reported the detection of 0.5 pg of PVY in purified preparations by DIBA (dot-immunobinding assay). The DIBA was rapid to perform and was as sensitive as DAS-ELISA in detecting CTV (citrus tristeza virus) in crude preparations, but it was possible only with the monoclonal antibodies. When polyclonal antibodies were used, nonspecific reactions with healthy controls prevented differentiation between positive and negative samples (Rocha-Pena *et al.* 1991). Smith *et al.* (1991) successfully detected BWYV and BMYV in *M. persicae* and *M. euphorbiae* caught in water traps in a crop of sugar beet, using the monoclonal antibodies and an amplified ELISA. The virus - carrying

aphids were not all infective, possibly because the virus was located in the gut and had not reached the salivary glands, which is a prerequisite for infectivity.

Our results showed that the sensitivity of both methods for detection of PPV is similar. Both clearly detected PPV in plant tissues but neither were able to detect virus particles in aphids.

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