Detection of Strains of Potato Virus X and of a Broad Spectrum of Potato Virus Y Isolates by Nucleic Acid Spot Hybridization (NASH)

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ABSTRACT

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Cloned complementary DNA (cDNA) hybridization probes for potato virus X (PVX) and potato virus Y (PVY) have been evaluated for the detection of PVX and PVY by nucleic acid spot hybridization (NASH). Results with four probes for PVX tested indicated a genomic difference among two Andean and two non-Andean strains. Three of the four PVX probes would be useful for detection of PVX strains. Results with two probes for PVY tested indicated specificity of the probes to the broad spectrum of 27 PVY isolates from the PVY^O, PVY^N, and PVY^C groups of strains obtained from different geographical areas. None of the PVY probes detected genomic differences among the PVY groups of strains. The PVY probes provided conclusive evidence that the other potyviruses tested, namely, potato virus A (PVA), potato virus V (PVV), Peru tomato virus (PTV), wild potato mosaic virus (WPMV), and tobacco etch virus (TEV), are viruses distinct from PVY. The two PVY probes would be useful for detection of PVY strains.

Baulcombe et al (2–4) described a nucleic acid spot hybridization method (NASH) for the detection of potato virus X (PVX), potato virus Y (PVY), and potato leafroll virus in crude sap samples. The procedure is based on a method of Owens and Diener (15) for viroid detection and involves the hybridization of a ^{32}P-labeled complementary DNA (cDNA) probe with virus in crude sap spots previously immobilized on a nitrocellulose membrane. They found that NASH was as good as or better than enzyme-linked immunosorbent assay (ELISA) in terms of sensitivity and reproducibility and permitted the handling of a larger number of samples. Thus, it is likely that this method will find application in PVX and PVY detection in quarantine, seed certification, and breeding programs for resistance.

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To be a reliable test, the virus specificity of the hybridization probes and their ability to detect a broad spectrum of strains need to be evaluated. In this work, hybridization probes for PVX have been tested against distinct PVX strains. Probes for PVY have been similarly evaluated against a broad spectrum of PVY isolates obtained from different geographical areas and against other potyviruses known to infect potato or other natural hosts of PVY.

MATERIALS AND METHODS

The preparation, labeling, hybridization, and autoradiography procedures for cDNA probes specific to each virus were essentially as described by Baulcombe et al for PVX (3). The cDNA was prepared from a PVX isolate of strain-group 3 (6) and a British common strain of PVY^O (8) and cloned using standard procedures (2,3). Four different probes for PVX and two for PVY have been tested. Two microliters of each crude sap sample were spotted onto nitrocellulose filters (Schleicher & Schull BA 85, 0.45 μm) that had been wetted in water, followed by equilibration in 3 M NaCl, 0.3 M trisodium citrate (20 × SSC). The filters were then air-dried and baked in vacuo at 80 C for 2 hr. The autoradiographs were scored visually.

Isolates of PVX and of PVY and other potyviruses were maintained in *Nicotiana glutinosa* L. and *N. occidentalis* L.,

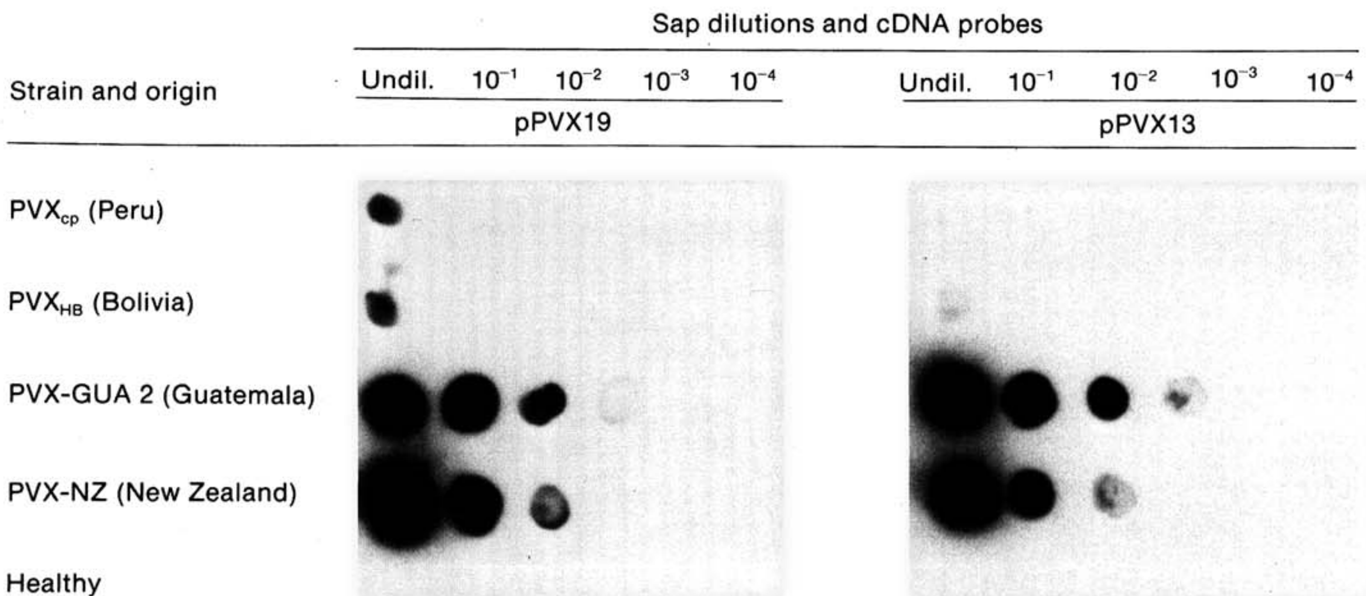

Fig. 1. Detection of PVX strains by nucleic acid spot hybridization. Several dilutions of crude sap from PVX-infected and healthy *Nicotiana* plants (2 μl each) were immobilized on nitrocellulose, hybridized with each of two ^{32}P-labeled cloned cDNAs (pPVX19 and pPVX13), and autoradiographed.

respectively. PVX (14) is a PVX_{cp} common strain in Peru and belongs to strain-group 2 (6); PVX_{HB} from Bolivia differs from other known strains of PVX in not inducing necrotic local lesions in inoculated leaves of *Gomphrena globosa* L. and in readily infecting PVX-immune clones (14). PVX-GUA 2 from Guatemala is not detected by antibodies to PVX_{cp} in latex tests, and in ELISA the reaction is weak (J. Abad, International Potato Center, *personal communication*). PVX-NZ was isolated in New Zealand from the cultivar Ilam Hardy, which is resistant to PVX (J. Fletcher, Private Bag Christchurch, *personal communication*).

Sap from infected or healthy leaves was extracted and used fresh or previously frozen for 24 hr at −20 C. The same crude sap samples used for NASH were used for ELISA diluted 1:9 in phosphate buffered saline + 0.05% Tween 20 and 2% polyvinylpyrrolidone (M_r 44,000). The direct double antibody sandwich form of ELISA as described by Clark and Adams (5) was used.

All virus propagation, sap extraction, and ELISA work was carried out at the International Potato Center. Nitrocellulose filters were mailed to Cambridge, England, and processed there for hybridizations.

Table 1. Identity of isolates shown in Figure 2

No.	Isolate[a]	Origin[b]
1	T (PVY^O)	Peru, La Molina
2	SF (PVY^O)	Peru, La Molina
3	255 (PVY^O)	Peru, La Molina
4	220 (PVY^O)	Peru, Canete
5	224 (PVY^O)	Peru, Canete
6	H13 (PVY^O)	Peru, Cajamarca (S)
7	2 (PVY^O)	Chile
8	171 (PVY^O)	Chile
9	189 (PVY^O)	Chile
10	52 (PVY^O)	Ecuador
11	CC5 (PVY^N)	Peru, Cuzco (S)
12	140 (PVY^N)	Peru, Cuzco
13	201 (PVY^N)	Peru, Cuzco
14	240 (PVY^N)	Peru, Cuzco
15	198 (PVY^N)	Peru, Huanuco
16	15.4 (PVY^N)	Peru, Huancayo
17	15.7 (PVY^N)	Peru, Huancayo
18	37692 (PVY^N)	Peru, Huancayo
19	129 (PVY^N)	Chile
20	133 (PVY^N)	Chile
21	8 (PVY^N)	Argentina
22	36 (PVY^N)	Argentina
23	38 (PVY^N)	Argentina
24	48 (PVY^N)	Argentina
25	2 C (PVY^C)	Eucador
26	85 N (PVY^C)	Ecuador
27	Y^C-Arran (PVY^C)	Holland (B)
28	PTV	Peru, La Molina
29	PVY^C-GL (PVV)	Holland (B)
30	PVY^C-AB (PVV)	Northern Ireland (C)
31	UF (PVV)	Peru (F)
32	TEV	United States (Fu)
33	PVA-1	Germany
34	PVA-5	Chile
35	PVA-327	Holland (B)
36	PVA-328	Holland (B)
37	WPMV	Peru (F)
38	Healthy	...
39	Healthy	...
40	Healthy	...

[a] PVY = potato virus Y, PTV = Peru tomato virus, PVV = potato virus V, TEV = tobacco etch virus, PVA = potato virus A, WPMV = wild potato mosaic virus.

[b] E. N. Fernandez-Northcote supplied all strains except those marked (B), (C), (F), (Fu), or (S). (B) = J. A. De Bokx, Plant Protection Institute, Wageningen, Holland; (C) = R. Copeland, Department of Agriculture, Belfast, Northern Ireland; (F) = C. E. Fribourg, Universidad Nacional Agraria, La Molina, Lima, Peru; (Fu) = R. W. Fulton, University of Wisconsin, Madison; (S) = L. F. Salazar, The International Potato Center, Lima, Peru.

RESULTS

All isolates used were positive in ELISA when tested with their corresponding antibodies.

Four probes for PVX (pPVX1, pPVX8, pPVX13, and pPVX19) (3) have been tested against the distinct PVX strains. Three showed the same general pattern, shown in Figure 1 for pPVX19, of hybridizing with all strains but more strongly with GUA 2 and NZ than with cp and HB. One probe (pPVX13) did not hybridize with the cp strain and showed only a weak reaction with HB, the other Andean strain (Fig. 1).

Two probes for PVY (pPVY6 and pPVY15) have been tested against a broad spectrum of 27 PVY isolates from the common (PVY^O), necrotic (PVY^N), and C (PVY^C) groups of strains (8), obtained from different geographical areas, and against other potyviruses known to infect potato or other natural hosts of PVY. Both probes hybridized only with the various PVY isolates from the O, N, and C groups of strains (Fig. 2; Table 1). The PVY probes either did not hybridize or showed only a weak reaction with the other potyviruses tested. A duplicate filter processed in parallel with the data shown in Figure 2 confirmed that the faint marks close to samples 28, 35, and 39 were not bona fide hybridization signals.

DISCUSSION

A previous report describing the use of NASH for the detection of PVX (3) suggested that this method would be more specific than ELISA using polyclonal antibodies. This is confirmed here by the observation that strains PVX_{HB} and PVX_{cp}, both of which can be detected with PVX polyclonal antibodies, are not detected by the PVX13 probe. Both show a slight hybridization reaction with the other PVX probes, however. This is consistent with the results obtained by Torrance et al (17) using monoclonal antibodies, which showed that these two strains form a serological group distinct from the main group of PVX strains. We should note, however, that a strong hybridization reaction is not always associated with serological cross-reactivity, as shown by the hybridization reaction with PVX-GUA 2, which is serologically distinct from the main group of PVX strains (and from PVX_{HB} and PVX_{cp}).

Because PVX_{HB} and PVX_{cp} are both Andean strains, it will be interesting to determine the strain specificity of probes prepared with the respective viral RNAs and to see whether this correlates with the geographical origin of PVX strains.

Previous serological tests showed that the PVY^O, PVY^N, and PVY^C groups of PVY strains are closely related and cannot be easily distinguished (7,10). Gugerli and Fries (12) produced a monoclonal antibody (MA C-9) that reacted in direct ELISA with 24 PVY isolates from the three groups of strains. This MA C-9 also reacted in direct ELISA with all the PVY isolates used in this work except isolate PVY^C-Arran (10).

Results with PVY probes indicated specificity of the probes to the broad spectrum of PVY isolates tested from the PVY^O, PVY^N, and PVY^C groups of strains. None detected genomic differences among the PVY groups of strains, which

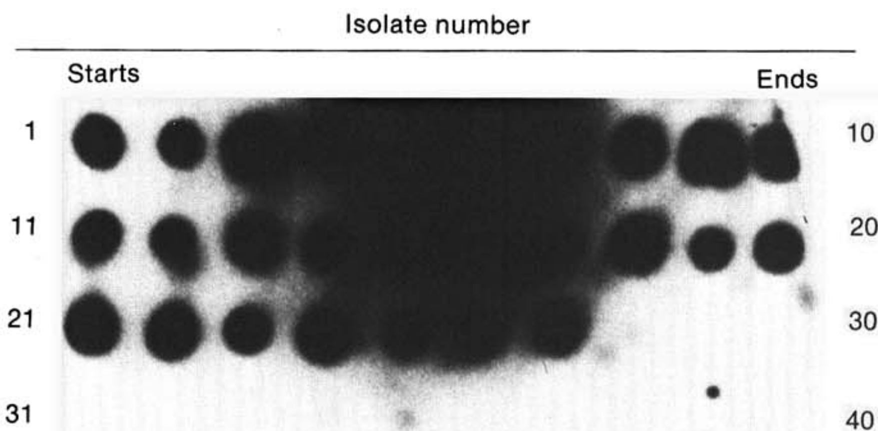

Fig. 2. Nucleic acid spot hybridization test with isolates from different PVY group of strains and related potyviruses. Crude sap (2 μl) from infected and healthy *Nicotiana* plants was immobilized on nitrocellulose, hybridized with ^{32}P-labeled pPVY15 cDNA, and autoradiographed. The isolates spotted in the membrane are identified in Table 1.

are differentiated on the basis of aphid transmission and symptoms induced on *N. tabacum* L. 'White Burley' (8).

These PVY probes provided conclusive evidence that the other potyviruses tested—PVA, PVV, PTV, WPMV, and TEV—are distinct from PVY. Previous serological evidence that took into account only the part of the genome responsible for their coat protein indicated they were different but distantly related (1,7,9–11,13,16).

The two PVY probes tested would be useful for detection of all PVY strains and can be obtained by application to D. C. Baulcombe. pPVY6 is approximately 2,400 base pairs and pPVY15 is 1,850 base pairs long. The two overlap by 1,000 base pairs at the 3′ of pPVY15 and the 5′ of pPVY6 (polarity as in the viral RNA). The region included in these probes extends between more than 3,250 bases in the central part of the PVY genome, outside the coat protein gene (Baulcombe, *unpublished*). Further work will use other clones derived from the rest of the PVY genome to detect any regions showing a greater or lesser ability to diagnose specific strains of PVY.

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