POTATO VIRUS A DETECTION BY REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION

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Summary. – Simple and reliable procedure for sample preparation and reverse transcription-polymerase chain reaction (RT-PCR) detection of potato virus A (PVA) is described. PVA-specific primers used in the RT-PCR defined a target sequence of 321 bp and did not produce amplification product(s) with potato virus Y.

Key words: potato virus A; NIb gene; reverse transcription; polymerase chain reaction; gel electrophoresis; Southern blot analysis

Introduction

PVA, firstly reported in 1932 (Murphy and McKay, 1932), is one of more than 50 viruses infecting potato. Although the occurrence of PVA is sporadic, this virus is spread worldwide in all potato-growing areas (Bartels, 1971). The main host of PVA is potato, but it could infect other species of *Solanaceae*, too (Brunt *et al.*, 1996). PVA alone causes in most potato cultivars mild mosaic symptoms only or the infection remains symptomless, however, coinfections with potato viruses X (PVX) and/or Y (PVY) which cause crinkle disease of potato are more serious and result in high decline of tuber yield (Bartels, 1971). PVA is not listed among the EPPO quarantine pests (Smith *et al.*, 1997), but because of its incidence with PVX and PVY, PVA is monitored in seed producing fields as well as in virus-free seeds produced by micropropagation cultures.

PVA is a member of genus *Potyvirus* and similarly to PVY, the N- and C- terminal domains of its coat protein are exposed on the surface of viral particles (Shukla *et al.*, 1988). These domains carry virus-specific antigenic markers which are easily removed during virus purification and therefore corresponding antisera without a cross-reactivity are difficult to prepare. Monoclonal antibodies could overcome this problem but their preparation is still very laborious (Čeřovská *et al.*, 1997).

In this paper, a method for PVA detection based on RT-PCR is described.

Materials and Methods

Host plants. PVA samples collected from various potato cultivars were used for mechanical infection of *Nicotiana tabacum* cv. Samsun plants (Table 1). Positive and negative control samples of PVA and PVY were from an enzyme-linked immunosorbent assay (ELISA) detection kit (Loewe Biochemica, Germany).

Sample preparation. One leaf disc of 6 mm diameter was homogenised in 500 µl of extraction buffer (0.5 mol/l Tris.HCl pH 8.3, 150 mmol/l ethylenediamine tetraacetate (EDTA) and 3 mmol/l sodium azide), centrifuged briefly and 1 µl of supernatant was subjected to RT-PCR.

RT-PCR was performed with the Access RT-PCR System (Promega, USA) as described by Petrzik and Svoboda (1997). Oligonucleotide primers were designed for the RNA-polymerase (NIb) gene on the basis of the complete PVA nucleotide sequence (EMBL database accession No. Z21670). The first cDNA strand was synthesised using oligonucleotide primer PVA1 (5'-TTCT

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Abbreviations: ELISA = enzyme-linked immunosorbent assay; PVA, PVX and PVY = potato virus A, X and Y; RT-PCR = reverse transription-polymerase chain reaction

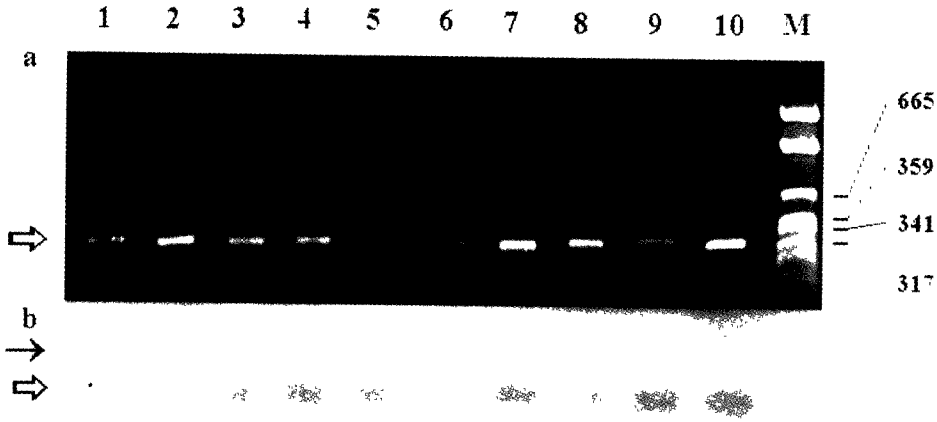

Fig. 1
Agarose gel electrophoresis (a) and Southern blot analysis (b) of amplification products of PVA samples
PVA samples from Table 1 (lanes 1–10). PBR322/*Dpn*I size markers (lane M, size in bp). Thick arrows mark the position of the 321 bp PVA-specific product. Thin arrow marks the position of the PVA-specific single-stranded amplification products on the blot.

TCATCCATTTGTTCATC-3') complementary to nucleotides (nt) 8511–8491. The PCR was performed with primer PVA2 (5'-GGT GAAATACTAATCCCAAAG-3') homologous to nt 8190–8211. The nucleotide numbering is according to Puurand *et al.* (1994). The primers were selected by use of the OLIGO Primer Analysis Programme (National Biosciences, Plymouth, MA, USA). The primers did not form dimers or stabile secondary structures and did not hybridise to each other. They amplified a target sequence of 321 nt of NIb gene. PVY-specific primers were designed for the coat protein gene and the 3'-nontranslated region, and produced a 260 bp amplicon (K. Petrzik, unpublished results). The reaction mixture contained besides the tested supernatant 2 U each of avian myeloblastosis virus (AMV) reverse transcriptase and *Tfl* DNA polymerase in AMV/*Tfl* reaction buffer, 0.2 mmol/l dNTPs, 1 mmol/l $MgSO_4$ and 20 nmoles of PVA1 and PVA2 primers. After 45 mins incubation at 42°C, the activity of the reverse transcriptase was destroyed by heating at 96°C for 3 mins. The amplification was done in 35 cycles (30 secs at 94°C, 1 min at 53°C and 2 mins at 68°C). Five µl of the amplification product was electrophoresed on 1.5 % agarose gel in a Tris-borate-EDTA buffer and DNA was stained with ethidium bromide.

Southern blot analysis. DNA probe was prepared from the amplified and purified PCR product of PVA-positive control from an ELISA kit. DIG DNA-Labelling and DIG DNA-Nucleic Acid Detection Kits (Boehringer, Germany), and random hexamers were used according to the manufacturer. Two µl of each PCR product was separated on 1.5% agarose gel, blotted overnight on a positively charged nylon membrane and hybridised with the PVA probe.

Results and Discussion

Ten tobacco plants in which the presence of PVA was confirmed previously by ELISA, and one positive and one negative control were subjected to PCR analysis (Table 1). In all the samples tested the PVA-specific 321 bp product was observed (Fig. 1a).

Table 1. List of potato cultivars tested

Cultivar	PCR	ELISA
Brno	+	+
IPO 410	+	+
Kardia	+	+
Karmen XVI	+	+
Lichte Industrie	+	+
Vltava	+	+
No. 6	+	+
No. 9	+	+
No. 11	+	+
No. 13	+	+
ELISA-positive control	+	+
ELISA-negative control	–	–

(+) = positive, (–) = negative.

The Southern blot analysis clearly confirmed PVA in all the samples, even though in those where the amount of the amplification product was on the detection treshold (Figs. 1a and 1b, lane 5). Faint bands of higher molecular mass of samples from cultivars Kardia and Lichte Industrie and sample No. 6 were visible on the blot but not on the gel. As they reacted with the probe, they were probably ssDNA artefacts of PVA origin (Fig. 1b, lanes 3, 5 and 7).

We tested the sensitivity of the RT-PCR assay by diluting a PVA-infected plant extract with an extract from healthy plants in a ratio of 1:5, 1:20, 1:50, 1:100 and 1:200. The dilution up to 200-fold which represented about 50 ng of

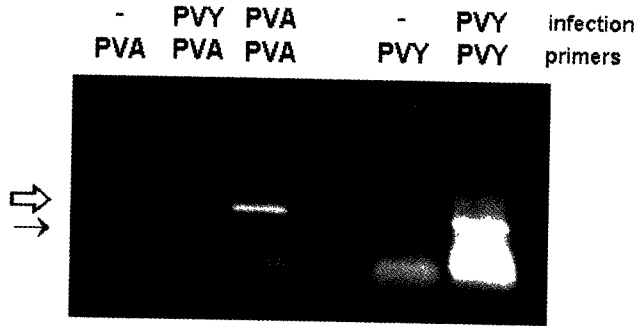

Fig. 2
Agarose gel electrophoresis of amplification products pf PVA and PVY samples
PVA- and PVY-specific primers used. Thick arrow marks the position of the 321 bp PVA-specific product. Thin arrow marks the position of the 260 bp PVY-specific product.

original PVA-infected plant material still resulted in a visible amplification product (data not shown).

PVA is known to be serologically related to PVY, tobacco etch and henbane mosaic potyviruses (Brunt *et al.*, 1996). Homology of up to 78 % was found in the coat protein gene of PVA and those of the papaya ringspot, pepper mottle, plum pox, sugarcane mosaic and tobacco vein mottling potyviruses, too (Puurand *et al.*, 1992). In potato-producing areas of central Europe coinfections with PVY are most probable (Maat and DeBokx, 1978).

The testing of the specificity of the PVA primers used on PVY-infected tobacco revealed that PVY did not produce band(s) with PVA1 and PVA2 primers (Fig. 2), but did produce a 260 bp band with PVY primers. Healthy plants did not give any amplification product either with PVA or PVY primers. We assume, therefore, that the primer set used in this study was PVA-specific.

Furthermore, the sample preparation method used was extremely simple and no additional nucleic acid purification was necessary in contrast to the procedure proposed by Singh and Singh (1997) which included DNase and proteinase K treatments and phenol extraction.

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