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Short Communication

Digoxigenin-labelled molecular probe for the simultaneous detection of three potato pathogens: potato spindle tuber viroid (PSTVd), potato virus Y (PVY), and potato leafroll virus (PLRV)*,**

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A molecular probe, p3POT, was constructed of PSTVd, PVY, PLRV cDNA fragments introduced into pUC18 vector. Sequencing of the inserts revealed that cloned fragments covered conservative parts of pathogenic genomes. Dot-blot hybridization of digoxigenin-labelled construct to crude extracts from plants infected with different potato viruses proved high sensitivity and specificity of the p3POT probe. This makes p3POT probe an useful tool for the routine testing, and selection of virus-free potatoes[1].

The economic importance of PSTVd, PVY, and PLRV necessitates intensive research on reliable and efficient methods for detection of those pathogens. Due to the lack of specific proteins [1], the method of choice for PSTVd detection is the dot-blot hybridization procedure [2, 3], and this technique can also be successfully applied for the detection of plant viruses [4]. In this paper we report the construction of the molecular probe, p3POT, containing PSTVd-, PLRV-, and PVY-specific inserts, and its application for simultaneous detection of those pathogens. For labelling of the probe a non-radioactive digoxigenin/AMPPD [3] system was chosen, which makes the reliability of the test comparable with that of ^{32}P-labelled probes.

MATERIALS AND METHODS

Construction of p3POT plasmid. PSTVd-specific cDNA insert (359 bp) from pAV401 plasmid [5] was recloned into *Bam*HI site of pUC18 vector, followed by recloning of PLRV cDNA (British isolate) from pLRV43 plasmid into *Sma*I site. Finally, PVY-specific cDNA fragment synthesized on a template of a British isolate of the common (PVY^{O}) PVY strain [4] was recloned into *Pst*I site from PVY15 plasmid according to standard procedures [6]. The resulting construct was purified from transformed competent *E. coli* DHα5-F′ cells by alkaline lysis procedure, analyzed by restriction

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** The nucleotide sequence of PVY cDNA fragment reported in this paper appeared in the EMBL nucleotide sequence database under the accession number Z29526.

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digestion and, after labelling, hybridization to cDNA inserts used for the construction (not shown).

Labelling of the probe. The p3POT plasmid was labelled with digoxigenin by incorporation of dig-11-UTP in "random primers" reaction [6].

Preparation of plant extracts for hybridization. Leaves of plants (healthy or infected) were ground with 1 ml/g of extraction buffer: 0.2 M Na_2HPO_4, 1% sodium lauryl sulfate (SDS), 5 mM dithiothreitol (DTT), 10 mM sodium diethyldithiocarbamate (DIECA), and the homogenate was pressed through the Miracloth tissue (Calbiochem-Behring Corp., La Jolla, U.S.A.). Denaturation and further steps were described previously [3]. The samples were applied on nylon membranes ZetaProbeGT (Bio-Rad).

Hybridization and digoxigenin detection. Hybridization conditions were the same as previously described [3], except that the prehybridization and hybridization solution contained: 50% formamide, 0.25 M NaCl, 0.12 M Na_2HPO_4 pH = 7.2, 7% SDS, 0.25 mg/ml salmon sperm DNA.

RESULTS AND DISCUSSION

Sequencing of the virus-specific fragments in the p3POT construct

PSTVd, PLRV, and PVY-specific cDNA fragments cloned into the p3POT construct were subjected to sequencing and their sequence compared with that of PSTVd [7], PLRV [8], and PVY [9, 10] RNAs. The results indicated high (92–99%) similarity of cDNA clones to all isolates of homologous pathogenic genomes sequenced so far. It should be stressed that the highest homology (99%) was found with the Polish isolate of PLRV. The sequence comparison indicated that all virus-specific cDNA fragments used as a molecular probe in this study covered highly conserved regions of pathogenic genomes, and should efficiently hybridize to a broad spectrum of different PSTVd, PVY, and PLRV isolates. This should be of importance in the attempts at selection of virus-free plants.

Detection of PSTVd, PVY, and PLRV with the p3POT probe

The digoxigenin-labelled molecular probe p3POT was used in the dot-blot hybridization detection of PSTVd, PVY, and PLRV in crude extracts from tomato, tobacco, and potato plants. The sensitivity of detection was tested by hybridization to diluted extracts from plants infected with PVY^{O}, mild (m) PSTVd, or PLRV (Fig. 1). For dilution extracts from healthy plants were used, which were also regarded as control samples in the hybridization (Fig. 1, lane 7). The results indicated that all pathogens were detectable in 1/256 – 1/1024 diluted extracts, whereas healthy extracts produced only a hardly visible signal on the blot. This makes testing of composite samples possible, which is of importance for phytosanitary practices.

To assess the specificity of detection the probe was hybridized to crude extracts from healthy plants, as well as from plants infected with PSTVd (mild and severe strains), PLRV, PVY (common and necrotic strains), Potato Virus X (PVX), Potato Virus S (PVS), and Potato Virus M (PVM). The results (Fig. 2) confirmed the sequence analysis (see above) and proved that the p3POT probe is suitable for specific detection of different strains of the pathogens. Moreover, no spurious signal was produced with

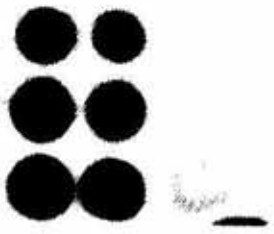

Fig. 1. Sensitivity of dot-blot hybridization detection of three potato pathogens: PSTVd, PVY, and PLRV with the digoxigenin-labelled p3POT probe.
Crude extracts from leaves of tobacco plants infected with PVY^{O}, tomato plants infected with mPSTVd, and potato plants infected with PLRV were diluted with extracts from healthy plants. Sample 1: undiluted extracts. Samples 2–6: dilutions 1/4, 1/16, 1/64, 1/256, and 1/1024, respectively. Sample 7: extracts from healthy plants.

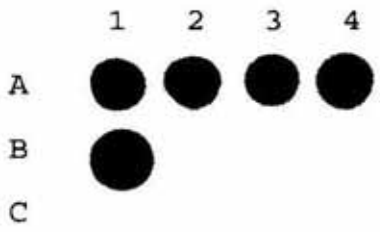

Fig. 2. Specificity of PSTVd, PVY, and PLRV detection with the digoxigenin-labelled p3POT cDNA probe.
The probe was hybridized to crude extracts from leaves of healthy plants (row C), and plants infected with different potato pathogens: row A — mPSTVd (sample 1), sPSTVd (sample 2), PVY^{O} (sample 3), PVY^{N} (sample 4), and row B — PLRV (sample 1), PVS (sample 2), PVM (sample 3), and PVX (sample 4). Row C, sample 1: extract from healthy tobacco, sample 2: from healthy potato, and sample 3: from healthy tomato plants.

heterologous viruses, or with extracts from healthy plants. Therefore, in our opinion, the p3POT plasmid should be recommended as a molecular probe for simultaneous detection of three potato pathogens: PSTVd, PLRV, and PVY, also in large-scale programmes. In practice such a probe could substantially reduce the cost of testing for selection of virus-free plants. The application of an efficient non-radioactive labelling technique with digoxigenin makes the probe safe in use and stable after labelling. This is an important factor when considering mass-scale detection of plant pathogens in phytosanitary stations.

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