Development of a Detection System for Viruses of Woody Plants Based on PCR Analysis of Immobilized Virions

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ABSTRACT

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The genomes of several viruses infecting woody host plants were found to serve as templates for the reverse transcriptase-polymerase chain reaction (RT-PCR) while still contained within their capsids as virions in solution. A detection system was developed in which the virions could be assayed by RT-PCR after recovery from solution by immobilization directly onto polypropylene or polystyrene surfaces. Components of crude plant extracts that could otherwise inhibit the RT-PCR analysis were washed away, and the immobilized virions could then be detected by the PCR reaction. Quantitative RT-PCR assays of virions bound non-specifically to plastic were compared to those in which the viruses were bound by specific antiserum-directed immunocapture. Binding to plastic was inhibited to a greater extent by extracts of some of the host plants than was immunocapture.

Additional keywords: antigen capture-PCR, apple mosaic virus, cherry leafroll virus, citrus tristeza virus, grapevine fanleaf virus, prune dwarf virus, Prunus necrotic ringspot virus.

In woody crop species, effective virus disease control is predicated upon the use of healthy materials. There is no direct remedial treatment for viral diseases once infection becomes established in orchards or vineyards. However, early detection, quantitation, and control of virus infections in woody host crops may be difficult due to low or variable virus titers, uneven viral distribution within the plant, and the colored, oxidizing, cross-linking nature of woody plant extracts (5,20,24,25).

The development of a reverse transcriptase-polymerase chain reaction (RT-PCR) assay (15) provided a virus detection system with the sensitivity to potentially overcome some of the aforementioned difficulties. But the general application of this approach

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to the detection of viruses in woody host samples is often blocked because components of the plant extract can inhibit the enzymes of the RT-PCR assay. To circumvent this problem in grape tissue, a nucleic acid purification procedure was developed to remove the inhibitory components of the tissue extract (24). However, this procedure is time-consuming and not amenable to the processing of large numbers of samples. Enzyme-linked immunosorbent assay (ELISA) does allow for the facile detection of virus directly from extracts of woody plants, but ELISA lacks the sensitivity required for the detection of these viruses, which occur in low concentrations in their woody hosts (3,11,26).

Immunocapture-PCR (IC-PCR) is a combination of techniques that allows for the detection of low concentrations of viruses in oxidizing plant extracts. Inhibitory plant constituents are washed from immobilized virions, and the viral genome is then prepared in situ to serve as a template for reverse transcription (12,13,21,22,29). However, this approach to virus immobilization requires the prior purification of virus for preparation of antiserum. Preparation of antiserum may not be practical, especially for incompletely characterized or difficult-to-purify viruses.

Here we describe a virus immobilization technique that does not require the use of an antiserum. Viruses from extracts of infected plants are nonspecifically bound directly to a solid support. After the washing away of the crude extract solution, these bound virions can be detected directly using RT-PCR analysis. The specificity of this direct binding-PCR (DB-PCR) assay is conferred by the virus-specific sequences encoded in the PCR primers. Because removal of the virus capsid is not necessary for the exposure of the viral genome to the initial reverse transcriptase stage of RT-PCR (3,21,30), immobilization, washing, and RT-PCR can be carried out sequentially in a single tube.

We have observed that polypropylene tubes not only bind virions, but also bind gamma globulin. We have used this capacity to simplify the IC-PCR procedure in parallel with DB-PCR, so that each procedure can be done completely in a single tube. This single-tube assay for IC-PCR was run in parallel with the DB-PCR assay in order to compare the utility of each in the analysis of viruses from extracts of woody plants. The comparison involved a panel of six viruses: cherry leafroll (CLRV) and grapevine fanleaf (GFLV) nepoviruses; apple mosaic (ApMV), prune dwarf (PDV), and Prunus necrotic ringspot (PNRSV) ilarviruses; and citrus tristeza closterovirus (CTV).

MATERIALS AND METHODS

Virus isolates and host plants. Viruses and hosts used included GFLV-100 (10) in grapevine (*Vitis vinifera* L.) and in *Chenopodium quinoa* Willd.; PNRSV isolate Fulton G (6) in cucumber (*Cucumis sativus* L.), and a PNRSV isolate in cherry (*Prunus avium* L. cv. Ukrainisch Griotte); a PDV isolate in peach (*Prunus persica* (L.) Batsch) and cucumber; CLRV-W8 (18) in walnut (*Juglans regia* L.) and *C. quinoa*; ApMV from American Type Culture Collection (ATCC, PV32) in cucumber and an isolate in rose (*Rosa dilecta* Rehd.) with mosaic symptom; and quick decline strain of CTV in orange (*Citrus sinensis* (L.) Osbeck) courtesy of J. Ghazanfari, California Department of Food and Agriculture. The ApMV-infected rose and all infected and healthy herbaceous plants were maintained in the greenhouse.

Oligonucleotide primers. Oligonucleotide primers for CLRV, CTV, GFLV, and PDV were designed based on their nucleotide sequences. The primers for CLRV were selected from the noncoding, homologous regions on RNAs 1 and 2 between nucleotides 35 and 374 with the following sequences: 5′ GCTGTCTTTCCAGCAGTCAAACTAAT 3′ (antisense primer) and 5′ GGCAGCGAGCTTCGAGCCGCGTAAGG 3′ (sense primer; EMBL Accession No. Z34265). The CTV primers were of the coat protein region, between nucleotides 400 and 699 with the sequences: 5′ TCAAACGTGTGTTGAATTTCCCAAGC 3′ (antisense primer) and 5′ AACGCCCTTGGAGTCTGGGGTAGGA 3′ (sense primer; 17). The primers for GFLV were of the coat protein gene region located at the 3′ end of RNA 2 between nucleotides 762 and 1,083, with the sequences: 5′ CCAAAGTTGGTTTCCCAAGA 3′ (antisense primer) and 5′ ACCGGATTGACGTGGGTGAT 3′ (sense primer; 27). PDV primers were of the coat protein gene region located on RNA 3 between nucleotides 1,812 and 1,984, with the sequences: 5′ TAGTGCAGGTTAACCAAAAGGAT 3′ (antisense primer) and 5′ ATGGATGCGATGGATAAAATAGT 3′ (sense primer; 1). The primers for ApMV were from the partial sequence of RNA 3 cDNA, and its sequences were 5′ CTTCGGACCATAGACATC 3′ (antisense primer) and 5′ GAATAGTGTTTCAGTATG 3′ (sense primer; courtesy of V. Pallas, CSIS, Spain). PNRSV primers from sequence information obtained from the 3′ end of RNA 3 were provided by J. Crosslin, Washington State University. The primers' sequences were between nucleotides 127 and 423, as follows: 5′ CTTGACCTGCAATATCCTACTCG 3′ (antisense primer) and 5′ AGACGTCGTGACAGACGTCGAAG 3′ (sense primer). All primers were synthesized by OLIGOS Etc. Inc., Wilsonville, Oregon.

ELISA. The $F(ab')_2$-ELISA technique (23) was carried out as described using polyclonal antisera raised in rabbits against ApMV, CLRV, and GFLV. For PDV, PNRSV, and CTV, the indirect-ELISA technique (16) was performed. Monoclonal ascites, Na70C9, for PNRSV was purchased from Agdia, Inc., and monoclonal ascites for PDV was provided by Ramon Jordan, USDA-ARS, Beltsville, MD. Polyclonal antisera for both PDV and PNRSV were produced in our laboratory. For CTV, anti-CTV polyclonal goat antisera (supplied by J. Ghazanfari, Central California Tristeza Eradication Agency, Tulare, CA) and anti-CTV polyclonal rabbit antisera (produced in our laboratory) were used.

Virus purification, RNA extraction, and sample preparation. The following methods were used to purify the viruses in this experiment: for GFLV in *C. quinoa,* as described by Savino et al (28); for PNRSV, PDV, and ApMV in cucumber, as per Crosslin and Mink (4) and Fulton (7); for CLRV in *C. quinoa,* as developed by Rowhani et al (25); and for CTV in orange as developed by Bar-Joseph et al (2). For all DB-PCR and IC-PCR experiments, a total of 50 μl of dilution buffer (500 mM Tris-Cl, pH 8.3, containing 2% PVP-40, 1% PEG 6000, 140 mM NaCl, and 0.05% Tween 20) containing 2 μl of purified virus sample (1 μg/ml) was added to each microcentrifuge tube or microplate well. Virus concentrations were estimated from absorbance at 260 nm, assuming an extinction coefficient of $A_{260} = 25$ being equal to RNA at 1 mg/ml and on the reported percentages of RNA for PDV, ApMV, PNRSV, CLRV, and GFLV as 14, 16 (9), 16 (8), 40 (14), and 33% (19), respectively. Therefore, the calculated virus concentrations of 1 mg/ml for PDV, ApMV, PNRSV, CLRV, and GFLV were A_{260} readings of 3.5, 4.0, 4.0, 10.0, and 8.2, respectively. RNA from purified GFLV preparations was phenol extracted and ethanol precipitated.

Plant extracts were prepared from young leaves of GFLV-infected grape, PNRSV-infected cherry, CTV-infected orange, ApMV-infected rose, CLRV-infected walnut, and bark scrapings from PDV-infected peach. Symptomatic leaves of the herbaceous hosts (*C. quinoa* or *Cucumis sativus*) were used. Plant tissues were extracted in dilution buffer (1:10, w/v) using a polytron homogenizer (Model PT3000, Brinkmann) and extracts were strained through miracloth. The resulting aqueous solutions were used for DB- and IC-PCR amplification.

DB-PCR. Sterile 0.5-ml polypropylene microcentrifuge tubes (Outpatient Services, Inc., Petaluma, CA) were loaded with 50-μl aliquots of plant extracts or purified virus solutions and incubated at 37 C for 60 min. After three washes with ELISA washing buffer (PBS [8.0 g of NaCl, 0.2 g of KH_2PO_4, 2.9 g of $Na_2HPO_4 \cdot 12H_2O$, and 0.2 g of KCl per liter, pH 7.4] containing 0.05% Tween 20), cDNA synthesis was done directly in the tubes (i.e., without any disruption treatment of virion particles) by adding 19.5 μl of reverse transcription solution (2 μl of 10× PCR buffer [500 mM KCl and 100 mM Tris-Cl, pH 9.0], 2 μl of 10 mM dNTP mixture [Pharmacia], 4 μl of 25 mM $MgCl_2$, also 0.5 μl of RNasin [40 units(U)/μl, Promega Corp.], 1 μl of antisense primer [200 ng/μl], 10 μl of sterile distilled water), and 0.5 μl of cloned MMLV-reverse transcriptase (200 U/μl, Promega). This mixture was overlaid with mineral oil, and tubes were placed

in a thermal cycler (Model 480, Perkin Elmer-Cetus) held at 37 C for 30 min. The reaction mixture was heated to 99 C for 2 min and then chilled for 2 min at 5 C. Amplification of the newly synthesized cDNA was followed by further addition to the reaction mixture of 79.5 μl of amplification solution (8 μl of 10× PCR buffer, 4 μl of 25 mM $MgCl_2$, 1 μl of sense primer (200 ng/μl), and 66.5 μl of sterile distilled water) and 0.5 μl of Taq polymerase (5 U/μl, Promega). The tubes were heated at 95 C for 2 min followed by 35 reaction cycles of 1 min at 99 C for melting, 1 min at 53 C for primer annealing, and 1 min at 72 C for primer extension. A final step at 72 C for 7 min was carried out prior to holding the samples at 4 C until removal from the thermal cycler. All PCR experiments described here and in subsequent sections were repeated two to three times for each test virus, and reproducible results were obtained.

IC-PCR. Sterile 0.5-ml polypropylene microcentrifuge tubes or polystyrene microplates (Costar Corp.) were precoated with 50 μl of purified antiviral IgG (1 mg/ml diluted [1:25] in carbonate buffer [1.59 g of Na_2CO_3, 2.93 g of $NaHCO_3$ per liter, pH 9.6]). The tubes or microplates were incubated for 60 min at 37 C. After three washes with ELISA wash buffer, 50-μl aliquots of plant extract or purified virus were added and incubated again for 60 min at 37 C and washed three times. cDNA synthesis and PCR were carried out as previously described for DB-PCR. However, with microplates, the transcription solution was transferred from the microplate wells to 0.5-ml polypropylene tubes following the 37 C–30 min incubation.

Standard RT-PCR. Complementary DNA synthesis and PCR amplification methods for standard RT-PCR were carried out as previously described for DB-PCR with one modification: 2 μl of purified virus sample were added to the reverse transcription cocktail to serve as the source of template for cDNA synthesis. The volume of sterile distilled water in the reverse transcription cocktail was reduced by 2 μl to adjust for the addition of virus sample to the reaction.

Analysis of the amplification products. Aliquots (10 μl) of each PCR reaction were analyzed by electrophoresis in 2% (w/v) agarose gel in TBE buffer (10.8 g of Tris · base, 5.5 g of boric acid, and 0.4 ml of 0.5 M EDTA per liter). Gels were electrophoresed at 5 V/cm for 90 min and stained with ethidium bromide (200 ng/ml) for 15 min. PCR products were visualized on a UV transilluminator and photographed using Polaroid 667 film.

Sensitivity of DB-PCR, IC-PCR, ELISA, and standard RT-PCR for virus detection. To determine the sensitivity of DB-PCR, IC-PCR, ELISA, and standard RT-PCR, purified virus solutions were serially diluted in sterile distilled water, added to dilution buffer (1:25 dilution; 50 μl final volume) and aliquotted into microcentrifuge tubes or microplate wells to achieve the following virus concentrations per reaction: 2 ng, 200 pg, 20 pg, 2 pg, 200 fg, 20 fg, and 2 fg. To determine the sensitivity of IC- and DB-PCR for detecting viruses in original hosts and alternate hosts, infected tissue extract (1:10, w/v) was mixed with healthy tissue extract (1:10, w/v), and fivefold serial dilutions were tested. The dilutions were made in healthy tissue extract to investigate the possibility of using DB- and IC-PCR in the detection of viruses from bulk samples collected from the field.

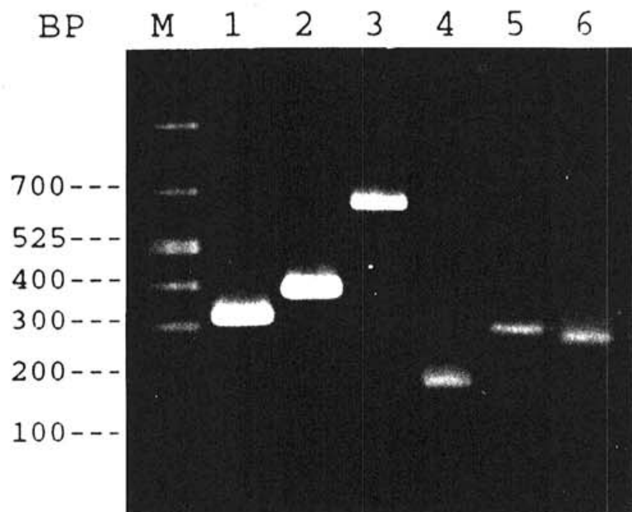

Fig. 1. Agarose gel electrophoretic analysis of IC-PCR amplified cDNA products from purified virus preparations. Lane 1, grapevine fanleaf virus; lane 2, cherry leafroll virus; lane 3, apple mosaic virus; lane 4, prune dwarf virus; lane 5, Prunus necrotic ringspot virus; and lane 6, citrus tristeza virus. Lane M, DNA markers ranging from 1,000 to 50 bp.

RESULTS

DB- and IC-PCR detection. Both DB- and IC-PCR consistently produced DNA products of the predicted sizes for all six viruses tested using purified or partially purified virus samples (Fig. 1), as well as crude homogenates of infected herbaceous and woody hosts (results not shown). Due to its restricted host range, CTV was not obtained from a herbaceous host, but only from citrus tissue. Specific product was not found in uninfected controls; no product was found in cases in which the PCR assay was attempted without the initial reverse transcriptase step.

Relative detection limits of DB- and IC-PCR. Tenfold serial dilutions of purified virus preparations were analyzed to establish the detection limits of DB- and IC-PCR tube assays for CLRV, GFLV, ApMV, PDV, and PNRSV. Results are summarized in Table 1. With DB-PCR, the detection threshold ranged from 20 to 200 pg per 100-μl reaction. For IC-PCR, it ranged from 20 to 200 fg per 100-μl reaction. Gel electrophoretic analysis of the PCR products for ApMV, for example, showed that PCR-amplified products were obtained with 20 pg of virus in the DB-PCR assay, while for IC-PCR in microcentrifuge tubes, it was 200 fg (Fig. 2, lanes 10 and 5, respectively).

Using a virus preparation of known concentration as a standard, we calculated that the minimum amount of GFLV we could detect as virions by standard RT-PCR was 20 fg per 100-μl reaction (Table 1). Removal of the coat protein from the virus by phenol extraction increased the sensitivity of the RT-PCR assay by a factor of two.

The PCR results using herbaceous and woody tissue extracts are summarized in Table 2. The detection limits of DB-PCR and IC-PCR were comparable when performed in microcentrifuge tubes in detecting PDV in peach extract, PDV and PNRSV in herbaceous host extracts, and CTV. However, DB-PCR was 25- to 625-fold less sensitive than IC-PCR in this assay for ApMV, CLRV, and GFLV in their herbaceous hosts, and for viruses isolated from cherry, walnut, rose, and grape. An example of the PCR results is illustrated in the gel electrophoretic analysis of the PNRSV amplified products (Fig. 3).

The sensitivity of the IC-PCR single-tube procedure was equal to that of the microplate well method, except for the detection of CTV, which exhibited a slight but reproducible decrease in sensitivity using the single-tube method. An example of the results

TABLE 1. Evaluation of the sensitivity of standard reverse transcriptase-polymerase chain reaction (RT-PCR), immunocapture-PCR (IC-PCR), direct binding-PCR (DB-PCR), and ELISA for the detection of five viruses

Virus[a]	Standard RT-PCR	IC-PCR	DB-PCR	ELISA
ApMV	200 fg[b]	200 fg	20 pg	2 ng
PDV	2 fg	20 fg	20 pg	2 ng
PNRSV	20 fg	20 fg	20 pg	200 pg
GFLV	20 fg	200 fg	20 pg	200 pg
CLRV	2 fg	200 fg	200 pg	2 ng

[a] ApMV = apple mosaic virus, PDV = prune dwarf virus, PNRSV = Prunus necrotic ringspot virus, GFLV = grapevine fanleaf virus, and CLRV = cherry leafroll virus.

[b] Values represent detection limits expressed as virus concentrations per PCR reaction (100 μl) or ELISA reaction (200 μl). Purified preparations were serially diluted in sterile distilled water.

in the comparison of the single tube and microplate methods using IC-PCR is seen in the gel electrophoretic analysis of PNRSV. Amplified products from cherry extract (Figs. 3 and 4) showed the limit of sensitivity to be at the 5^{-6} dilution (15,625×), using either microcentrifuge tubes or microplate wells (Fig. 3, lane 7, and Fig. 4, lane 7, respectively).

Comparison of DB-PCR and IC-PCR with other detection methods. The detection limits of ELISA and standard RT-PCR were determined using serial dilutions of purified virus samples for CLRV, GFLV, ApMV, PDV, and PNRSV. A comparison of the detection limits of ELISA, standard RT-, DB-, and IC-PCR in tubes is given in Table 1. Comparison of DB-PCR with ELISA showed a 10- to 100-fold improvement in detection sensitivity with the use of the PCR method; IC-PCR was 1,000- to 100,000-fold more sensitive than ELISA (an ELISA result is interpreted as positive if the A_{405} reading is two or more times higher than that of the healthy plant control). Comparison of RT-PCR with DB-PCR showed a 100- to 10,0000-fold increase in sensitivity for analysis of the virions directly in solution. RT-PCR was similarly more sensitive than IC-PCR, but by a smaller margin.

DISCUSSION

Although RT-PCR detection is four to six orders of magnitude more sensitive than ELISA for the viruses described here, phenolic

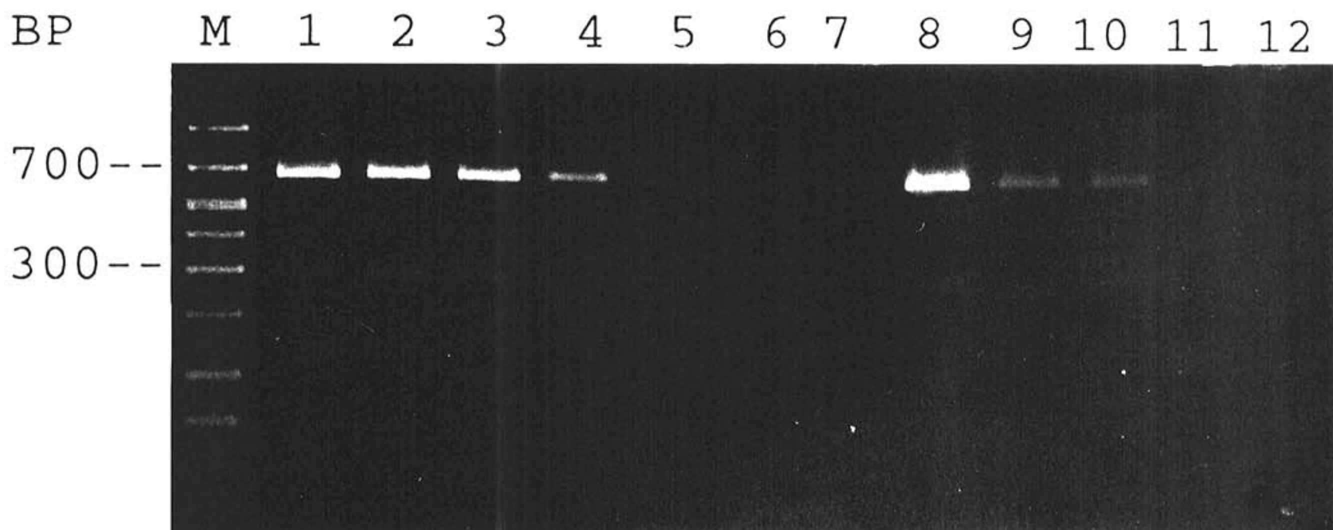

Fig. 2. Agarose gel electrophoretic analysis of apple mosaic virus cDNA products from IC-PCR tube assays (lanes 1–7) and DB-PCR (lanes 8–12). The following virus concentrations per reaction were used: lanes 1 and 8, 2 ng; lanes 2 and 9, 200 pg; lanes 3 and 10, 20 pg; lanes 4 and 11, 2 pg; lane 5, 200 fg; lane 6, 20 fg; lanes 7 and 12, negative buffer control. Lane M, DNA markers ranging from 1,000 to 50 bp.

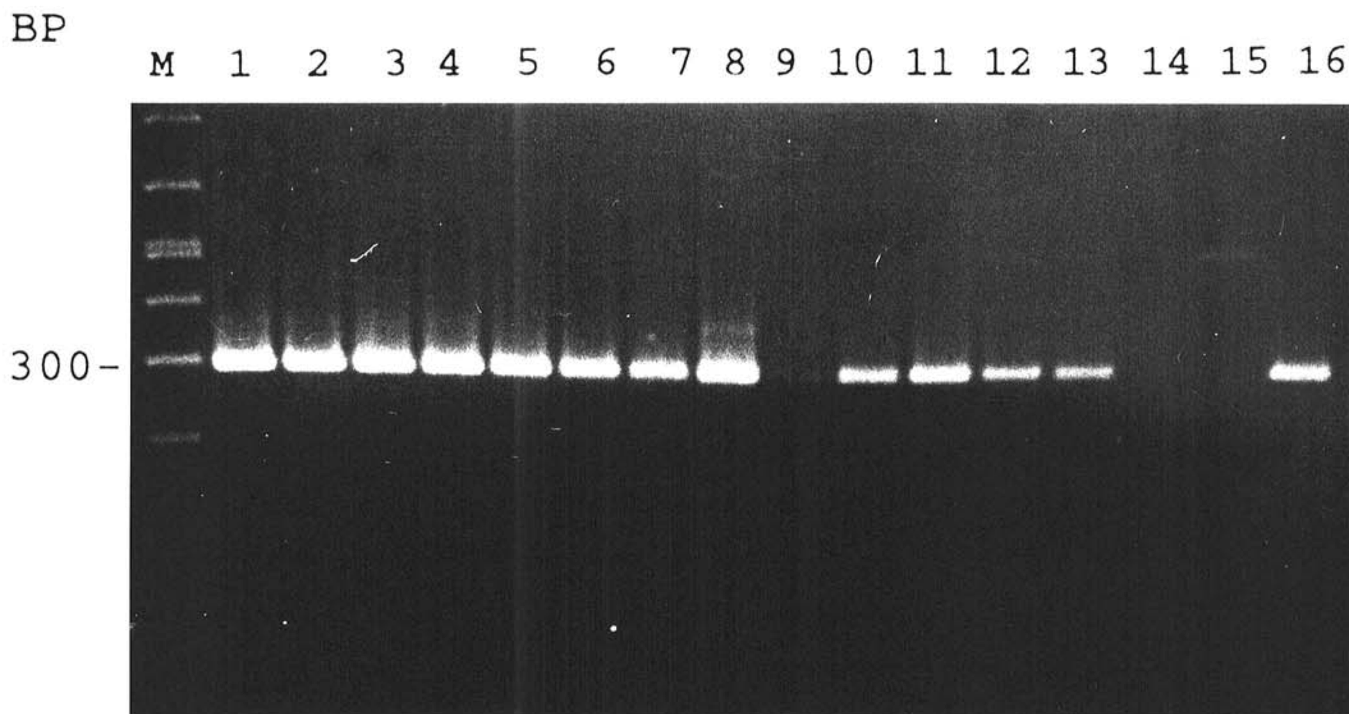

Fig. 3. Agarose gel electrophoretic analysis of Prunus necrotic ringspot virus cDNA products from IC-PCR tube assays (lanes 1–9) and DB-PCR (lanes 10–16) of infected *Prunus avium* leaf tissue. The following dilutions of infected tissue extract (1:10, w/v) diluted in healthy tissue extract (1:10, w/v) were analyzed: lanes 1 and 10, stock extract of infected tissue in dilution buffer (1:10, w/v); lanes 2 and 11, 5^{-1} dilution; lanes 3 and 12, 5^{-2} dilution; lanes 4 and 13, 5^{-3} dilution; lanes 5 and 14, 5^{-4} dilution; lane 6, 5^{-5} dilution; lane 7, 5^{-6} dilution; lanes 8 and 16, purified virus sample (2 μg per reaction); lanes 9 and 15, stock extract of healthy tissue in dilution buffer (1:10, w/v); and lane M, DNA markers ranging in size from 1,000 to 50 bp.

compounds, polysaccharide, and other inhibitory components of tissue extracts prevent its direct application to viruses from woody hosts (5,20,24,25). However, we have found that such inhibitors can be easily eliminated by immobilizing the virions to a solid support and washing. We have shown this in the comparison of two procedures, each of which removes inhibitory plant components to allow for the immediate application of RT-PCR analysis.

Use of the nonspecific virion immobilization system, i.e., DB-PCR, has the benefit that virus-specific antiserum is not required. The savings afforded by the independence of this system from the requirement of the production of antiserum is balanced, however, in that the detection levels achieved by DB-PCR were generally lower than those of immunocapture analysis. The reduction in sensitivity was most evident in tissue extracts of woody host plants. In these extracts, inhibitors of virus binding lowered detection levels by as much as 600-fold.

TABLE 2. Evaluation of immunocapture-PCR (IC-PCR) and direct binding-PCR (DB-PCR) in polypropylene microcentrifuge tubes for the detection of various viruses in plant tissue extracts

Pathogen[a]	Host plant	IC-PCR	DB-PCR
ApMV	*Cucumis sativus*	5^{-6}[b]	5^{-3}
ApMV	*Rosa dilecta*	5^{-3}	5^{-1}
PDV	*C. sativus*	5^{-6}	5^{-6}
PDV	*Prunus persica*	5^{-5}	5^{-6}
PNRSV	*C. sativus*	5^{-6}	5^{-6}
PNRSV	*Prunus avium*	5^{-6}	5^{-3}
GFLV	*Chenopodium quinoa*	5^{-6}	5^{-2}
GFLV	*Vitis vinifera*	5^{-6}	5^{-2}
CLRV	*C. quinoa*	5^{-6}	5^{-3}
CLRV	*Juglans regia*	5^{-3}	5^{-1}
CTV	*Citrus sinesis*	5^{-5}	5^{-5}

[a]ApMV = apple mosaic virus, PDV = prune dwarf virus, PNRSV = Prunus necrotic ringspot virus, GFLV = grapevine fanleaf virus, CLRV = cherry leafroll virus, and CTV = citrus tristeza closterovirus.
[b]Values represent end-point dilution involving fivefold serial dilutions of infected tissue extract (1:10, w/v) in healthy tissue extract (1:10, w/v).

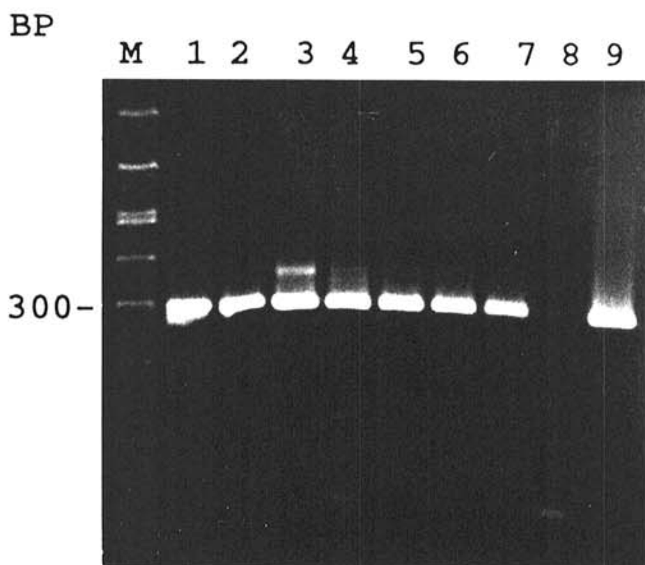

Fig. 4. Agarose gel electrophoretic analysis of Prunus necrotic ringspot virus cDNA products from IC-PCR microplate assays of infected *Prunus avium* leaf tissue. The following dilutions of infected tissue extract (1:10, w/v) diluted in healthy tissue extract (1:10, w/v) were analyzed: lane 1, stock extract of infected tissue in dilution buffer (1:10, w/v); lane 2, 5^{-1} dilution; lane 3, 5^{-2} dilution; lane 4, 5^{-3} dilution; lane 5, 5^{-4} dilution; lane 6, 5^{-5} dilution; lane 7, 5^{-6} dilution; lane 8, stock extract of healthy tissue in dilution buffer (1:10, w/v); lane 9, purified virus sample (2 μg per reaction); and lane M, DNA markers ranging from 1,000 to 50 bp.

The IC-PCR technique detected all test viruses in femtogram ranges, demonstrating that the sensitivity of immunocapture allows virus detection at concentrations well below those detectable by ELISA. To simplify the previously described IC-PCR procedure (21), the single-tube format developed for DB-PCR was adapted. All steps of IC-PCR, including virus immobilization and PCR analysis, were carried out in the same tube (e.g., polypropylene microcentrifuge tubes), as described for the detection of human hepatitis A virus (13). The single-tube assay involved fewer manipulations than IC-PCR in microplate wells, thus lowering the risk of sample contamination and allowing the testing of much larger sample numbers. This method has been previously described for the detection of plum pox virus in infected apricots (29). Our results indicate that IC-PCR in a single tube was as sensitive as IC-PCR in microplate wells.

Standard RT-PCR analysis of virus in solution was the most sensitive detection system for samples free of PCR inhibitors found in woody host plants. However, the inhibitory substances contained in woody hosts could not be totally eliminated by diluting the tissue extract. Increased sensitivity of direct RT-PCR compared to IC-PCR (Table 1) was likely due to the inefficiency of complete recovery of virus from solution by immunocapture. We calculated that viral genomic RNA was directly accessible as template for the RT-PCR reactions in 50% of the virus particles in solution.

The PCR-based detection techniques described here simplify the methods currently used for the detection of woody host viruses, while dramatically increasing the level of sensitivity over that at which viruses are currently detected. This approach should benefit virus elimination programs by facilitating the analysis of small amounts of tissue generated in meristem cultured plants and in large scale testing of inventories from clean stock programs throughout the growing season. As the techniques of molecular biology become more common, the synthesis of PCR primers based on double-stranded RNA-derived cDNA sequence data will become more prevalent. Thus, future research on a widening range of viruses may well rely for specificity on sequence-specific primer binding instead of on specific antiserum and may employ DB- and IC-PCR for studies in epidemiology and host-pathogen interactions.

ADDENDUM

Since acceptance of this paper, ApMV (ATCC PV32) was found to react with antiserum against PNRSV.

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