

## A simple and rapid method for processing tissue infected with plum pox potyvirus for use with specific 3' non-coding region RT-PCR assays<sup>1</sup>

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A rapid, simple method for preparing plant tissue infected with plum pox potyvirus (PPV) using a commercial product known as Gene Releaser is described. The Gene Releaser polymeric matrix method produces plant extracts suitable for PCR amplification without the use of organic solvents, ethanol precipitation, or additional nucleic acid purification techniques. We also describe the development of a PPV-specific amplification assay based on the unique 220 nucleotides present in the 3' non-coding region of the PPV genome. This paper demonstrates the simplicity of the Gene Releaser method combined with the accuracy of the PCR assay for the detection of multiple PPV strains from Spain, France, Greece, Italy, Germany, Egypt, Hungary, and Romania. Amplification of the 3' non-coding region of potato Y potyvirus (PVY) using primers for the 3' non-coding region of the PVY genome was also possible with Gene Releaser preparation of viruliferous *Myzus persicae* to demonstrate the potential usefulness of this work for PPV detection from aphids.

### Introduction

Since the first report of plum pox potyvirus (PPV) in Bulgaria in the 1930s (Atanasoff, 1932, 1934), PPV has spread steadily through Europe, the Mediterranean, and in the late 1980s to Egypt (Mazyad *et al.*, 1992). Recently, PPV was identified in Northern India and Chile (Thakur *et al.*, 1992; Acuña, 1994). The continued spread of PPV into countries where it has not been previously reported is of great concern to the quarantine efforts of the USA where it remains, as of this writing, unreported.

The polymerase chain reaction (PCR) technology has become the most powerful tool for plant pathologists since the adaptation of ELISA to plant virology (Clark & Adams, 1977). PCR has made a pronounced impact in the area of detection and identification of plant viruses and other pathogens (Hadidi *et al.*, 1995). We, and others, have developed PCR assays for the rapid and sensitive detection of PPV from *Prunus* spp. (Korschineck *et al.*, 1991; Levy & Hadidi, 1991; Wetzel *et al.*, 1992). However, many of these protocols involve lengthy procedures for sample preparation, and are based on the non-specific coat-protein region of the PPV genome. With the recent detection of Asian prunus latent potyvirus (APLV) in *Prunus* germplasm which reacts positively with PPV coat-protein primers, in Southern blot hybridization with a PPV coat-protein clone, and in ELISA with PPV polyclonal antiserum (Hadidi & Levy, 1994), we were faced with the need to modify our existing PPV PCR detection method to obtain a more rapid and PPV-specific assay. To that end, we have applied a commercially available product for the rapid preparation of *Prunus* samples and viruliferous aphids for PCR assays.

PPV PCR has been possible from *Prunus* tissue. Occasionally, we and others have experienced difficulties during amplification of nucleic acids from *Prunus* tissue. Many of

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these plant tissues harbor inhibitors of enzymatic reactions involved in reverse transcription (RT) and/or PCR which require procedural modifications to remove such inhibitors. Published PPV PCR protocols have included sample preparations such as: the treatment of diluted sap with proteinase K (Korschineck *et al.*, 1991); preparation of nucleic acids by phenol/chloroform extraction followed by column chromatography (Levy & Hadidi, 1991); concentration and immobilization of target virus with polyclonal or monoclonal antibodies (Wetzel *et al.*, 1992). These additional procedures result in increased expense and labour per sample, not to mention the generation of hazardous waste. The preparation of *Prunus* and aphid samples using the Gene Releaser (GR)<sup>1</sup> polymeric matrix (BioVentures Inc., Murfreesboro, US) offers several advantages including the use of small tissue amounts, avoidance of organic solvents and, most importantly, low cost and rapid sample preparation.

To improve the accuracy of PPV detection in our laboratory we focused on the development of a PPV-specific PCR protocol based on the unique 3' non-coding region maintained by isolates of PPV. The accuracy of our test relies on the sequence of the 3' non-coding region of potyviruses which serves as the molecular basis for identification and distinction among members of the potyvirus group (Ward & Shukla, 1991). Amongst isolates of the same potyvirus, the 3' non-coding region displays sequence homologies between 83 and 99%, yet different potyviruses exhibit sequence variability at levels of 39–53% (Frenkel *et al.*, 1989, 1992; Ward & Shukla, 1991; Van der Vlugt *et al.*, 1993). PPV and potato Y potyvirus (PVY) primers were designed based on observations of the high degree of sequence homology within the PPV 3' non-coding region following examination of published sequence information (Maiss *et al.*, 1989; L  n *et al.*, 1989; Teycheney *et al.*, 1989; Wetzel *et al.*, 1991; Cervera *et al.*, 1993; Palkovics *et al.*, 1993). Similarly, PVY primers were designed because of the high degree of sequence homology at the 3' non-coding region of several isolates of PVY (Robaglia *et al.*, 1989; Van der Vlugt *et al.*, 1989, 1993). PPV 3' non-coding region primers were used to develop a PPV-specific PCR assay for use with various tissues of *Prunus* spp., and PVY 3' non-coding region primers were used to develop a PVY-specific PCR assay from the aphid *Myzus persicae*. PVY was chosen for aphid experiments due to the conditions which restrict the use of PPV-viruliferous aphids in Beltsville (US) in accordance with the APHIS permit under which PPV research is conducted. In addition, both PPV and PVY are transmitted by the same vector in a non-persistent manner.

This paper describes the preparation of samples for PCR using the GR polymeric matrix and the development of a PPV-specific 3' non-coding region PCR assay that when combined provide a simple, rapid, and specific PCR assay for the detection of PPV. A detailed description of the Gene Releaser protocol and its utility for the detection of various pathogens has been reported (Levy *et al.*, 1994).

## Materials and methods

### *Source of virus isolates, insects, and PPV clone*

PPV (American Type Culture Collection PV-286) was maintained in tobacco (*Nicotiana benthamiana*) at the USDA quarantine facility in Beltsville (US). The following PPV isolates were provided by M. Ravelonandro (INRA, Bordeaux, FR) and were inoculated and maintained in *Prunus tomentosa* by V. Damsteegt (USDA-ARS, Frederick, US) at the USDA containment facility: isolate D from France; isolate 286 from Germany; isolate M

<sup>1</sup> Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the products by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable. This article reports the results of research only.

reactions involved in reverse transcription to remove such inhibitors. Published procedures such as: the treatment of diluted samples; preparation of nucleic acids by phenol extraction (Levy & Hadidi, 1991); concentration of nucleic acids by ethanol precipitation; use of monoclonal antibodies (Wetzel & Hadidi, 1991); increased expense and labour per sample, not to mention the preparation of *Prunus* and aphid samples (BioVentures Inc., Murfreesboro, US) offers high yields and amounts, avoidance of organic solvents and the need for sample preparation.

In our laboratory we focused on the development of a specific 3' non-coding region maintained by the sequence of the 3' non-coding region of the virus for identification and distinction among isolates (Hadidi, 1991). Amongst isolates of the same species, sequence homologies between 83 and 99%, yet amino acid levels of 39–53% (Frenkel *et al.*, 1989, 1992; Hadidi, 1991). PPV and potato Y potyvirus (PVY) primers were used because of sequence homology within the PPV 3' non-coding region (Maiss *et al.*, 1991; Hadidi *et al.*, 1991; Cervera *et al.*, 1993; Palkovics *et al.*, 1993). Because of the high degree of sequence homology between isolates of PVY (Robaglia *et al.*, 1989; Van der Auwera *et al.*, 1991) primers were used to develop a PPV-specific PCR assay from the aphid *Myzus persicae*. PVY and PPV are transmitted by the same vector in a

PCR using the GR polymeric matrix and the 3' non-coding region PCR assay that when combined with the detection of PPV. A detailed description of the detection of various pathogens has been

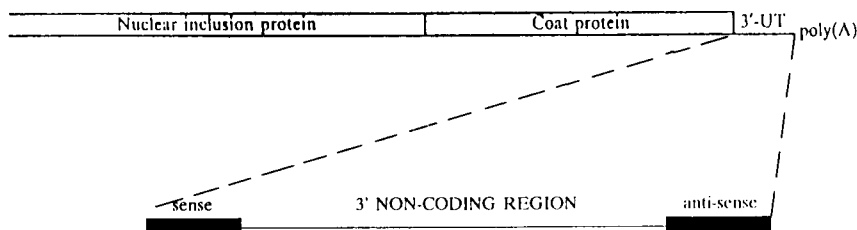
was maintained in tobacco (*Nicotiana glauca*, Beltsville (US). The following PPV isolates were provided by M. Kölber (Soil Conservation and Plant Protection Service, Budapest, HU) and were inoculated and maintained in tobacco (USDA-ARS, Frederick, US) at the following locations: isolate 286 from Germany; isolate M

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from Greece; isolate Salon de Provence from France; isolate El Amar from Egypt; and an isolate from Spain. PPV field isolates from Hungary in anthers from peach, apricot, and plum (*Prunus domestica*) were provided by M. Kölber (Soil Conservation and Plant Protection Service, Budapest, HU). PPV isolate N23 in plum from Italy was provided by M. Barba (Institute of Plant Pathology, Rome, IT). PPV isolate 24821 from Romania in GF305 peach was provided by H. Waterworth (USDA-ARS, Glenn Dale, US). PVY-infected tobacco (*Nicotiana tabacum*) was provided by J. Foster (USDA-APHIS, Beltsville). Aphids (*Myzus persicae*) were provided by R. Goth (USDA-ARS, Beltsville).

### Sample preparation

Plant samples were prepared according to the Gene Releaser (GR) manufacturers' recommendations with some modifications. P-1000 pipet tips were inverted (large end) and pressed into leaf tissue to form a leaf disk (approximately 30 mg) for processing in 1.5-ml microfuge tubes. This method was used to sample tissue which contained PPV in infected leaf tissue. About 10–20 anthers were processed per tube for PPV detection in anthers, and 3, 6, 9 or 12 insects per tube were processed for PVY detection in *M. persicae*. Collected tissues were ground with a disposable pestle (Kontes, Vineland, US) in 100  $\mu$ l (25  $\mu$ l for insect-containing tubes) per sample of ice-cold TE buffer (10 mM Tris-HCl, 1 mM ethylenediamine tetraacetic acid, pH 8.0) and 0.4 mg of carborundum per  $\mu$ l of buffer (120 grit, Buehler, Lake Bluff, US). Samples were then centrifuged (12,000 rev min<sup>-1</sup>) at 4°C for 1–2 min. The supernatants were removed to sterile 1.5-ml microfuge tubes and placed on ice. One-microlitre aliquots of each sample were placed in thin-walled PCR tubes (Perkin-Elmer Cetus, Norwalk, US) containing 23  $\mu$ l of freshly resuspended GR. The GR-extract mixtures were vortexed at low speed for 30 s, and held on ice until all the samples were prepared. The samples were then placed in a microwave-safe rack (polypropylene, BioVentures Inc.), overlaid with 50  $\mu$ l of mineral oil, lid closed, and microwaved (according to the manufacturers' recommendations) at the high power setting



### 3' NON-CODING REGION PRIMERS:

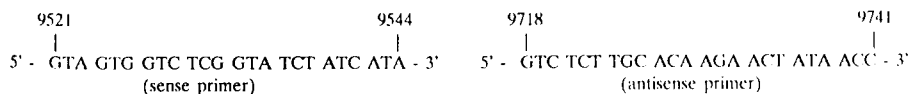


Fig. 1. Plum pox potyvirus 3' non-coding region utilized for the development of a PPV-specific PCR assay. Sense and antisense primer sequences are included.

Zone non codante 3' du plum pox potyvirus, utilisée pour le développement d'un test PCR spécifique au virus. Les séquences 'sense' et 'antisense' sont présentées.

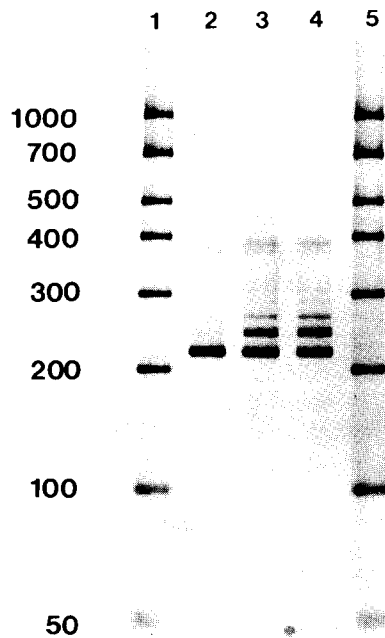
for a period such that heating time multiplied by the oven power rating (watts) equalled  $4500 \text{ W min}^{-1}$ .

#### Primers sequences and expected product size

Primers for PPV 3' non-coding region (Fig. 1) were based on the PPV sequence of Maiss *et al.* (1989) and generated a product 220 bp in size. Primers for PVY 3' non-coding region were the 25mer antisense primer 5' GTCAGTCTATGACAGAATCACAG 3', and the 24mer sense primer 5' GTGATGTAGTGTCTCTCCGGACGA 3' based on the PVY-N sequence of Van der Vlugt *et al.* (1989, 1993) which generated a product 269 bp in size.

#### RT-PCR amplification of the PPV or PVY 3' non-coding region

A 10–20  $\mu\text{l}$  aliquot of GR matrix containing sample was removed immediately after micro-waving (without cooling) and added to a primer annealing reaction mixture containing: 6  $\mu\text{l}$  of 5X first strand buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, and 15 mM  $\text{MgCl}_2$ ), 3  $\mu\text{l}$  of 0.1 M dithiothreitol (DTT), 1  $\mu\text{g}$  complementary primer and sterile  $\text{H}_2\text{O}$  to a final volume



**Fig 2.** Polyacrylamide gel electrophoretic analysis of PCR products of the 3' non-translated region of plum pox potyvirus from *Nicotiana benthamiana* leaf tissue prepared with and without Gene Releaser (GR). Lanes 1 and 5, BioLow DNA molecular weight markers; lane 2, 220-bp product from PPV-infected tobacco prepared with GR and microwave heating; lane 3, as lane 2 but without GR; lane 4, as 2 but without GR or microwave heating.

Analyse en électrophorèse sur gel polyacrylamide des produits PCR de la zone 3' non traduite du plum pox potyvirus, préparé avec ou sans Gene Releaser (GR) à partir de tissus foliaires de *N. benthamiana*. Voies 1 et 5 – marqueurs BioLow de poids moléculaire d'ADN; voie 2 – produit à 220 paires de bases obtenu par GR et traitement à micro-ondes à partir de tabac contaminé par le PPV; voie 3 – comme 2 mais sans GR; voie 4 – comme 2 mais sans ni GR, ni traitement à micro-ondes.

the oven power rating (watts) equalled

based on the PPV sequence of Maiss *et al.* primers for PVY 3' non-coding region were the 5'-AGAATCACAG 3', and the 24mer sense primer 3' based on the PVY-N sequence of Van der Vliet *et al.* product 269 bp in size.

**3' non-coding region**

Sample was removed immediately after microcentrifugation. The annealing reaction mixture containing: 6  $\mu$ l of 10X Taq polymerase buffer, 375 mM KCl, and 15 mM MgCl<sub>2</sub>, 3  $\mu$ l of primer and sterile H<sub>2</sub>O to a final volume

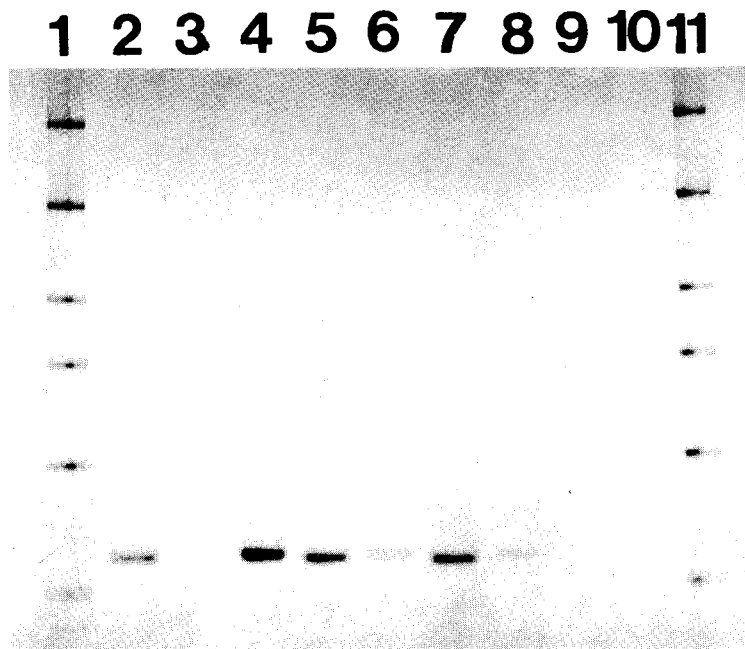


products of the 3' non-translated region of plum pox virus compared with and without Gene Releaser (GR). Lane 2, 220-bp product from PPV-infected tobacco prepared without GR; lane 4, as 2 but without GR or

its PCR de la zone 3' non traduite du plum pox de tissus foliaires de *N. benthamiana*. Voies 1 et 2 - produit à 220 paires de bases obtenu par GR et le PPV; voie 3 - comme 2 mais sans GR; voie 4

of 30  $\mu$ l. Sample-primer mixtures were vortexed briefly and denatured by heating at 100°C for 5 min, chilled on ice for 2 min and primers annealed at room temperature for 30-45 min. The annealed reaction was added to 20  $\mu$ l of a cDNA reaction mixture containing: 4  $\mu$ l of 5X first strand buffer, 2  $\mu$ l of 0.1 M DTT, 1  $\mu$ l of RNasin (40 units, Promega Corp., Madison, US), 5  $\mu$ l of 0.3 M 2-mercaptoethanol, 2.5  $\mu$ l of 10 mM dNTPs (2.5 mM each dGTP, dATP, dTTP and dCTP), and 1  $\mu$ l of Maloney murine leukemia virus (200 U  $\mu$ l<sup>-1</sup>) reverse transcriptase (Promega Corp.). Reactions were mixed briefly, and incubated for 1-1.5 h at 42°C.

Amplifications were performed in thin-walled PCR tubes and contained the following reaction mixture: 5  $\mu$ l of 10X PCR buffer (1X is 10 mM Tris-HCl, pH 8.3, 50 mM KCl, and 0.001% gelatin), 3  $\mu$ l of 25 mM MgCl<sub>2</sub> (1.5 mM final concentration), 1  $\mu$ l of 10 mM dNTPs, 1  $\mu$ l each of 6  $\mu$ M complementary and homologous DNA primer, 2.5 units of DNA Taq polymerase (Promega) and sterile H<sub>2</sub>O to a volume of 45  $\mu$ l. The reaction mixtures were overlaid with 75  $\mu$ l of mineral oil and 'hot-started' (Chou *et al.*, 1992) at 85°C in a DNA thermocycler (Perkin-Elmer Cetus). After 5 min at 85°C, 5  $\mu$ l of the GR-cDNA mixture was added to the PCR reaction and amplified with the following cycling parameters: denaturation at 94°C for 30 s, primer annealing at 62°C for 30 s (55°C for PVY primers), and extension at 72°C for 45 s for 30 cycles with a final extension at 72°C for 7 min.



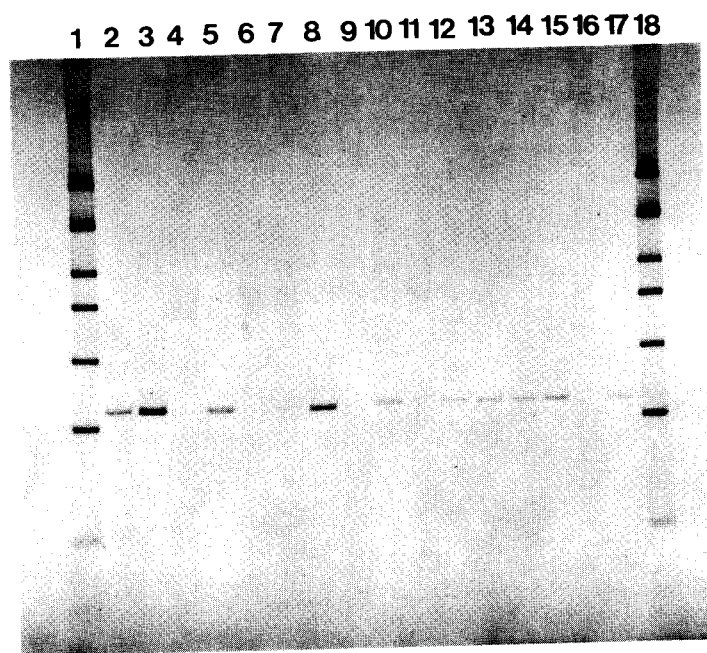
**Fig. 3.** Polyacrylamide gel electrophoretic analysis of PCR products of the 3' non-coding region of PPV from various PPV strains prepared with GR. Lanes 1 and 11, BioLow DNA markers; lane 2, PPV-286 from Germany; lane 3, healthy *Prunus tomentosa*; lane 4, PPV from Spain; lane 5, PPV-Salon de Provence from France; lane 6, PPV-M from Greece; lane 7, PPV-D from France; lane 8, PPV-El Amar from Egypt. Analyse en électrophorèse sur gel polyacrylamide des produits PCR de la zone 3' non traduite de diverses souches du plum pox potyvirus, préparés par GR. Voies 1 et 11, marqueurs ADN BioLow; voie 2, PPV-286 d'Allemagne; voie 3, *P. tomentosa* sain; voie 4, PPV d'Espagne; voie 5, PPV-Salon de Provence de France; voie 6, PPV-M de Grèce; voie 7, PPV-D de France; voie 8, PPV-El Amar d'Égypte.

*Electrophoretic analysis of PCR products*

Following amplification, RT-PCR products were electrophoresed in 5% native polyacrylamide gels at 150 V for 1.5 h in 1X TBE (89 mM Tris, 89 mM borate, and 2.5 mM Na<sub>2</sub>EDTA, pH 8.3) and visualized by staining with silver nitrate. Product sizes were determined using the BioLow DNA molecular weight markers (BioVentures, Inc.).

**Results and discussion**

An inexpensive, simple, and rapid sample preparation involving the application of a commercial product was adapted for use with the 3' non-coding region PCR detection assays of PPV and PVY from plant tissue and insect vectors, respectively. Intractable tissues such as peach, apricot, and plum harbor inhibitors of RT and/or PCR enzymatic reactions which may require additional purification and/or procedural modifications for the elimination of these inhibitors. The use of Gene Releaser polymeric matrix for preparing plant and aphid tissue extracts proved an effective method that did not require the use of phenol and chloroform, ethanol precipitation or lengthy and laborious extraction procedures. The manufacturers' suggested use of TE buffer worked well in our hands for samples containing viral nucleic acids.



**Fig. 4.** Polyacrylamide gel electrophoretic analysis of PCR products of the 3' non-coding region of PPV from anther tissue collected from a cultivar collection in the field in Hungary prepared with GR. Lanes 1 and 18, BioLow DNA markers; lanes 2-8, anthers of apricot cultivars; lanes 9-14, anthers of peach cultivars; lanes 15-17, anthers of plum cultivars.

Analyse en électrophorèse sur gel polyacrylamide des produits PCR de la zone 3' non traduite du PPV, préparés par GR à partir d'étamines prélevées sur une collection hongroise de cultivars. Voies 1 et 18, marqueurs ADN BioLow; voies 2-8, étamines de cultivars d'abricotier; voies 9-14, étamines de cultivars de pêcher; voies 15-17, étamines de cultivars de prunier.

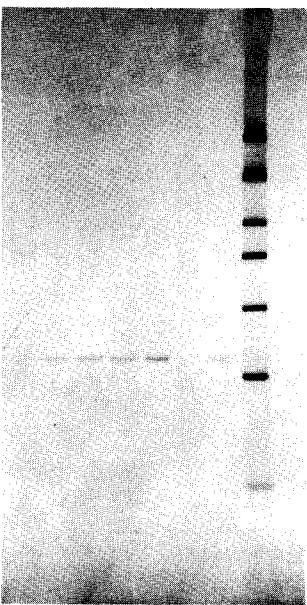
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electrophoresed in 5% native polyacrylamide (BioLob) containing 0.1 M borate, and 2.5 mM Na<sub>2</sub>EDTA, pH 8.3) and fragment sizes were determined using the BioLob (BioLob).

preparation involving the application of a modified protocol for the 3' non-coding region PCR detection of PPV using specific vectors, respectively. Intractable tissues were processed using a series of RT and/or PCR enzymatic reactions and several procedural modifications for the elimination of the polymeric matrix for preparing plant and aphid samples. This did not require the use of phenol and chloroform and laborious extraction procedures. The method worked well in our hands for samples containing

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products of the 3' non-coding region of PPV from samples collected in Hungary prepared with GR. Lanes 1 and 18, BioLob DNA markers; lanes 2-5, 9, and 12, aphids per assay tube, respectively.

produits PCR de la zone 3' non traduite du PPV, extraits de la collection hongroise de cultivars. Voies 1 et 18, marqueurs ADN BioLob; voies 2-5, 9 et 12, pucerons par analyse, respectivement.

Woody plant viruses often reach higher titers in experimental herbaceous host plants. PPV in one of its tobacco hosts, *N. benthamiana*, was amplified with and without GR tissue preparation. Fig. 2 shows the results of RT-PCR of PPV from *N. benthamiana* using primers specific for the 3' non-translated region of PPV. A 220-bp product was observed. Lane 2 represents PPV-infected tobacco tissue prepared with GR. Lane 3 represents PPV-infected tobacco prepared by sap dilution in TE buffer without GR and subjected to microwave heating. Lane 4 represents PPV-infected tobacco prepared as in lane 3 but without microwave heating. However, without the use of GR, an increased number of non-specific bands are present along with the 220-bp product suggesting mis-priming possibly caused by some interference of host components in either cDNA or PCR reactions, or both (Fig. 2, lanes 3 and 4).

The results of RT-PCR using GR for *Prunus* sample preparation and the PPV 3' non-coding region primers for strain amplification are shown in Fig. 3. The expected 220-bp product was observed for several isolates of PPV including: PPV-286 from Germany (lane 2), PPV from Spain (lane 4), PPV-D from France (lane 5), PPV-M from Greece (lane 6), PPV-Salon de Provence from France (lane 7), and PPV-El Amar from Egypt. Lanes 4-8 were maintained in *P. tomentosa*. Lane 3 represents healthy *P. tomentosa*.

Despite restrictions on the movement of *Prunus* budwood, the movement of anther tissue may serve as a source of germplasm and is therefore a quarantine concern in certain countries. Anther tissue from field trees of peach, plum, and apricot in Hungary was tested using the GR and PPV 3' non-coding region assay. Fig. 4 demonstrates the association of PPV with *Prunus* anther tissue. Lanes 2-8 represent apricot cultivars, lanes 9-14 represent peach cultivars, and lanes 15-17 represent plum cultivars. Based on this result we conclude that unrestricted

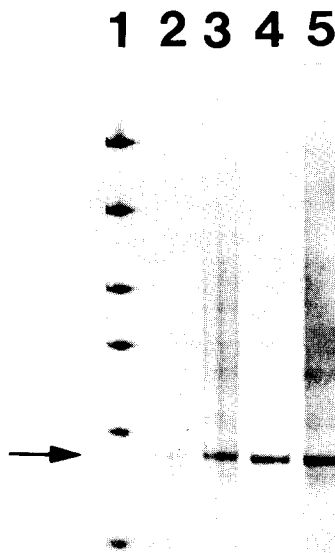


Fig. 5. Polyacrylamide gel electrophoretic analysis of PCR products of the 3' non-coding region of PVY from viruliferous *Myzus persicae*. Lane 1, BioLob DNA markers; lanes 2-5, 3, 6, 9, and 12 aphids per assay tube, respectively.

Analyse en électrophorèse sur gel polyacrylamide des produits PCR de la zone 3' non traduite du PVY extrait de *M. persicae* virulifères. Voie 1 - marqueurs ADN BioLob; voies 2-5 - 3, 6, 9 et 12 pucerons par analyse, respectivement.

movement of *Prunus* anthers as germplasm for breeding programs may prove to be a source of PPV introduction into countries currently PPV-free.

Because of the US restriction for conducting experiments involving PPV transmission by insect vectors, we decided to test the utility of GR tissue preparation, and PVY 3' non-coding region RT-PCR for the detection of the PVY genome in the aphid *Myzus persicae*. Fig. 5 shows the PCR amplification of the PVY 3' non-coding region from 3, 6, 9, and 12 aphids ground immediately following a 1-2 min aphid acquisition feeding period on PVY-N infected tobacco leaves (lanes 2-5, respectively).

According to the manufacturer, the GR polymeric matrix binds inhibitors. Once bound, these inhibitors do not interfere with enzymatic reactions even when the matrix is present in RT-PCR and PCR reactions. We have, however, experienced difficulties amplifying cDNA preparations stored frozen in the GR matrix. Removal of the remaining GR matrix by centrifugation and removal of cDNA to a clean microfuge tube prior to freezer storage of cDNA solved this problem.

The use of GR in the preparation of samples for PCR offers a simple, rapid, and inexpensive alternative to current methods. In our hands, about 20 samples can be available for PCR and RT-PCR in 1-2 h versus 1-2 days for tissues containing viruses. This product application is cost-effective and results in rapid sample preparation, and should result in the increased utility of PCR for large-scale sampling, geographic pathogen surveys, pathogen epidemiology studies, and detection of pathogens in their insect vectors which may have previously been hampered because of lengthy sample extraction methods.

In addition to the simple and rapid preparation of tissue, we have increased our specificity of PPV detection with the utilization of the unique PPV 3' non-coding region sequence for the construction of PCR primers. Amplification of this region eliminates the cross amplification of genes which share greater sequence homology within members of the potyvirus group. Such was the case during an investigation of peach from China and *Prunus mume* from Japan thought to contain PPV due to RT-PCR results using PPV coat-protein primers and hybridization results using a PPV coat-protein clone which misidentified Asian prunus latent potyvirus as PPV in Asian *Prunus* (Hadidi & Levy, 1994). Finally, we have achieved additional sensitivity and specificity by exploiting the 3' non-coding region to develop a PPV-specific clone useful in PCR dot-blot detection. This clone does not hybridize to Asian prunus potyvirus.

#### Acknowledgements

This work was supported in part by a USAID-NARP grant administered by OICD/USDA to A. Hadidi.

#### Une méthode simple et rapide de préparation de tissus contaminés par le plum pox potyvirus en vue de leur analyse par PCR-RT à base de la région non codante 3' spécifique du virus

L'article décrit une méthode rapide et simple pour le conditionnement des tissus de plantes contaminés par le plum pox potyvirus (PPV) à l'aide d'un produit commercial, le Gene Releaser. La méthode, à matrice polymérique, permet de préparer des extraits végétaux en vue de l'amplification PCR sans recours aux solvants organiques, à la précipitation à l'éthanol, ou autre technique de purification des acides nucléiques. Est présenté aussi le développement d'une technique d'amplification spécifique du PPV, basée sur les 220 nucléotides uniques présents dans la zone non codante 3' du génome du virus. La méthode Gene Releaser apporte une grande simplicité, qui se combine à la spécificité du test PCR, tel que nous l'avons appliqué à la détection de diverses souches du PPV d'Espagne, France, Grèce, Italie, Allemagne, Egypte,

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xperiments involving PPV transmission by tissue preparation, and PVY 3' non-coding region in the aphid *Myzus persicae*. Fig. 5 shows region from 3, 6, 9, and 12 aphids ground feeding period on PVY-N infected tobacco

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PCR offers a simple, rapid, and inexpensive t 20 samples can be available for PCR and aining viruses. This product application is on, and should result in the increased utility en surveys, pathogen epidemiology studies, which may have previously been hampered

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RP grant administered by OICD/USDA to

on de tissus contaminés par le par PCR-RT à base de la région non

le conditionnement des tissus de plantes l'aide d'un produit commercial, le Gene et de préparer des extraits végétaux en vue organiques, à la précipitation à l'éthanol, ou . Est présenté aussi le développement d'une e sur les 220 nucléotides uniques présents La méthode Gene Releaser apporte une est PCR, tel que nous l'avons appliqué à la France, Grèce, Italie, Allemagne, Egypte,

Hongrie et Roumanie. Dans le cas du PVY, la méthode Gene Releaser a permis l'amplification de la zone non codante 3' à partir de *Myzus persicae* virulifères, indiquant l'utilité éventuelle de la méthode pour détecter le PPV dans les pucerons.

**Быстрый и простой метод обработки ткани, зараженной plum pox potyvirus, для подготовки к анализу методом обратной транскрипции полимеразной цепной реакции в специфической некодирующей 3'-концевой области**

Описывается быстрый и простой метод подготовки ткани, инфицированной plum pox potyvirus (PPV) с помощью товарного препарата, известного под названием Gene Releaser. В используемом методе с помощью полимерной матрицы препарата Gene Releaser приготавливаются растительные экстракты, предназначенные для использования в амплификации полимеразной цепной реакцией без использования органических растворителей, спиртового осаждения или применения других методов пурификации нуклеиновых кислот. Описывается разработка методики амплификации, специфичной к PPV, основанной на типичном наборе 220 нуклеотидов, присутствующих в некодирующей 3'-концевой области генома PPV. В публикации показывается простота метода с использованием препарата Gene Releaser в сочетании с точностью метода полимеразной цепной реакции для детектирования множественных штаммов вируса PPV, обнаруженного в Испании, Франции, Греции, Италии, Германии, Египте, Венгрии и Румынии. Амплификация некодирующей 3'-концевой области potato Y potyvirus (PVY) с использованием праймеров для некодирующей 3'-концевой области генома вируса PVY также представляется возможной благодаря препарату Gene Releaser для вирулентного вида *Myzus persicae*, демонстрируя потенциальную пользу проведенной работы для детектирования вируса PPV у тли.

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