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Accurate identification of plum pox potyvirus and its differentiation from Asian prunus latent potyvirus in *Prunus* germplasm[1]

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We have determined the accuracy of plum pox potyvirus (PPV) identification in reverse transcription-polymerase chain reaction (RT-PCR) assays using DNA primers for the 3' non-coding region of PPV as compared with primers for a segment of PPV coat protein. Only primers for the 3' non-coding region were specific for PPV identification. Primers for PPV coat protein reacted with other known potyviruses such as potato Y potyvirus and papaya ringspot potyvirus. The 3' non-coding region sequence of PPV has great value in the specific differentiation of PPV from other potyviruses. PPV is the only potyvirus so far known to infect *Prunus* spp. We have now identified a latent virus, which we have named Asian prunus latent potyvirus (APLV), that infects germplasm of peach and *Prunus mume*. PPV can be differentiated from APLV by RT-PCR using DNA primers specific for the 3' non-coding region of the PPV genome. APLV, however, reacts positively with PPV coat-protein primers in RT-PCR assay, PPV cDNA probe of cloned PPV cDNA containing the coat protein gene in molecular hybridization assays, and PPV antiserum in serological assays.

Introduction

Plum pox potyvirus (PPV) is the only potyvirus known to infect stone fruits (Kegler & Shade, 1971; Németh, 1986; Dunez & Sutic, 1988). PPV causes the most devastating viral disease of these crops, because of reduced fruit quality, premature dropping of fruit, wide host range in cultivated and wild *Prunus* spp., rapid natural spread by aphid vectors, and rapid decline and death of trees when co-infected with other viruses (Németh, 1986).

Serological and molecular methods for identification of PPV by ELISA, molecular hybridization, and reverse transcription-polymerase chain reaction (RT-PCR) assays have utilized reagents and DNA primers derived from the PPV coat-protein gene (Adams, 1978; Dosba *et al.*, 1986; Varveri *et al.*, 1987, 1988; Korschineck *et al.*, 1991; Levy & Hadidi, 1991; Wetzel *et al.*, 1992). These methods may not be PPV-specific because sequence analysis of the PPV coat-protein gene has revealed considerable homology with the coat-protein gene of several other potyviruses (Laín *et al.*, 1989; Maiss *et al.*, 1989; Teycheney *et al.*, 1989; Wetzel *et al.*, 1991; Cervera *et al.*, 1993). The nucleotide sequence of PPV, however, contains a unique 220 nucleotide sequence at the 3' non-coding region adjacent to the coat-protein gene which is conserved in all PPV isolates sequenced (Laín *et al.*, 1989; Maiss *et al.*, 1989; Teycheney *et al.*, 1989; Wetzel *et al.*, 1991; Cervera *et al.*, 1993) and differs from the 3' non-coding region of tobacco etch (TEV), tobacco vein mottling, pepper mottle, and sugarcane mosaic potyviruses (Maiss *et al.*, 1989), as well as other potyviruses. Thus, the 3' non-coding region may be used to identify PPV and differentiate it from other potyviruses. Here we report the accuracy of PPV identification in RT-PCR assays using DNA primers for the 3' non-coding region of PPV as compared with primers for a segment of PPV coat protein and the identification of a previously unreported virus, which we name Asian prunus latent potyvirus (APLV), in peach and Japanese apricot (*Prunus mume*) from eastern Asia.

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Materials and methods

ELISA

Direct ELISA (Clark & Adams, 1977) was used to assay leaf tissue. Assay volumes were 150 µl per well. Maxisorp microplates (A/S Nunc., Kamstrup, DK) were coated with polyclonal antiserum to PPV provided by J. Dunez (Kerlan & Dunez, 1979) diluted 1:1000 in 0.05 M sodium carbonate, pH 9.6, coating buffer and incubated at 4 °C overnight. Plates were washed 6-8 times between each step with water. Prior to sample addition, plates were blocked for 1 h at room temperature with PBS-Tween containing 0.2% non-fat milk powder. Samples were ground (1:10 w/v) in PEP buffer [PBS-Tween, 0.2% ovalbumin, and 2% polyvinylpyrrolidone (PVP), pH 7.4] and incubated at 4 °C overnight. Alkaline phosphatase-conjugated PPV antiserum was added at a dilution of 1:1000 and incubated at 37 °C for 3 h. Plates were washed 8-10 times with water prior to the addition of p-nitrophenyl phosphate (pNPP) (Sigma, St Louis, US) at a concentration of 1 mg ml^{-1} in substrate buffer (9.7% diethanolamine, pH 9.8). Colour was developed overnight at room temperature.

ELISA results were visualized spectrophotometrically at 405 nm with a Multiscan microplate reader (TiterTek, Labsystems, FI). Positives were the mean of at least four wells per plate having a value of at least 2.5 times of healthy uninfected control mean values.

cDNA synthesis and PCR amplification

Primers for PPV coat-protein region were a 20-mer primer (5' GCGATTAACATCAC-CAGCGG-3') complementary to PPV-NAT RNA nucleotides 9469-9488 (Maiss *et al.*, 1989) (antisense primer) and a 20-mer primer (5'-GCGAGACTATGATGTCACGG-3') homologous to PPV-NAT RNA nucleotides 9014-9033 (Maiss *et al.*, 1989) (sense primer). Primers were designed to give a PPV DNA fragment of 474 bp in RT-PCR assays. Primers were synthesized by Synthecell Inc., Rockville (US). Total nucleic acids were extracted from leaf tissue as described by Yang *et al.* (1992). Nucleic acid preparations were further purified using RNase-free ELUTIP-r minicolumns (Schleicher and Schuell, Keene, US) as described by Hadidi *et al.* (1993). PPV cDNA was synthesized and amplified from the minicolumn-purified total nucleic acids by the following procedure. For each sample, deionized sterile water was added to a mixture of 1 µg total nucleic acids, 1 µg PPV antisense primer, 6 µl of 5X first strand cDNA buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM $MgCl_2$ and 50 mM dithiothreitol) to yield a final volume of 30 µl. The resulting mixture was denatured by heating at 100 °C for 5 min, chilled on ice for 2 min and annealed at room temperature for 1 h. The following reagents were then added to the reaction mixture: 4 µl 5X first strand cDNA buffer, 5 µl 300 mM 2-mercaptoethanol, 2.5 µl 10 mM dNTP (2.5 mM each of dATP, dGTP, dTTP and dCTP), 1 µl RNasin (40 units per µl, Promega Corp., Madison, US), 1.0 µl of cloned maloney murine leukemia virus reverse transcriptase (200 units per µl, GIBCO BRL Life Technologies, Inc., Gaithersburg, US), and deionized water to a volume of 50 µl. The reaction was incubated at 42 °C for 2.5 h. Aliquots (5 µl) of the resulting PPV cDNA were transferred to tubes each containing 45 µl of 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$ and 0.01% gelatin), 200 µM dNTP (dGTP, dATP, dTTP and dCTP), 1.2 µM each of the antisense and sense primers for the coat protein of PPV, 2.5 units of AmpliTaq DNA polymerase (Hoffman-LaRoche, Branchburg, US) and deionized water to a total volume of 50 µl. The reaction was overlaid with 75 µl of mineral oil to prevent evaporation during amplification. Cycling parameters were 1 min at 94 °C, 2 min at 55 °C, and 3 min at 72 °C for 30 cycles with final extension at 72 °C for 7 min in a DNA thermal cycler (Pekin-Elmer Cetus Corp., Norwalk, US).

Primers for PPV 3' non-coding region were a 24-mer primer (5'-GTCTCTTGCACAA-

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Results and discussion

During our screening for PPV by ELISA and by blot hybridization of tissues from quarantined peach originally imported by USA from China, we found some cultivars to be positive for PPV or a hitherto unreported potyvirus with PPV coat-protein antiserum and with a ^{32}P-labeled cRNA probe containing the viral coat-protein gene (Table 1). Similar results were obtained with some accessions of *P. mume* imported by Canada or the US from Japan (data not shown). These results were unexpected because PPV has not been reported previously from east Asia, and no other potyvirus is known to infect *Prunus*.

To determine the specificity of the PPV coat-protein region for PPV identification, we have utilized DNA primers derived from PPV coat-protein nucleotide sequences in RT-PCR assays to compare PPV amplification in nucleic acid extracts of infected tissue with that of potato Y potyvirus (PVY), the type member of the potyvirus group, and also cloned PPV DNA. Fig. 1 shows polyacrylamide gel electrophoretic and Southern blot hybridization analyses of the PPV coat-protein RT-PCR assay. A major DNA fragment of the expected size (474 bp) from cloned PPV DNA and from PPV or PVY-infected tissue was amplified (A, lanes 2, 4, 6 and 7) and hybridized with a ^{32}P-labeled cRNA probe containing PPV coat-protein gene (B, lanes 2, 4, 6 and 7). No amplified 474 bp DNA fragment or hybridization was obtained from uninfected tissue (A and B, lanes 3 and 5). Similarly, in dot-blot hybridization analysis of RT-PCR products amplified using PPV coat-protein primers (Fig. 2), the PPV cRNA probe hybridized with RT-PCR products of nucleic acid extracts from the east Asian germplasm (A, lanes 2-5) and PPV-infected tissue (B, lanes 1 and 2) but not with uninfected tissue (B, lane 3). The hybridization signals of the probe, however, were generally stronger with known PPV controls than with the virus(es) from east Asian germplasm, possibly suggesting differences in virus titre or in the nucleotide sequence of the coat-protein gene, or else that the virus(es) from east Asian germplasm may not belong to the potyvirus group. Thus, the virus(es) detected in peach and *P. mume* may be PPV, an undescribed potyvirus that infects *Prunus* spp. and/or a non-potyvirus that shares some homology with potyviruses.

To determine whether or not PPV was actually present in east Asian germplasm, we designed DNA primers specific for the PPV 3' non-coding region to generate a PPV-specific fragment of 220 bp by RT-PCR from nucleic acid extracts of PPV-infected tissue. Fig. 3 shows that a major DNA fragment of 220 bp was amplified from PPV cDNA clone (A, lane 2) and from stone fruit and *Nicotiana benthamiana* tissues infected with known PPV isolates (A, lanes 3-6 and B, lanes 2-4 respectively). No amplified products were observed from extracts of healthy tissue (B, lane 1) or tissues infected with several other potyviruses.

Table 1. Detection of a potyvirus from Chinese peach germplasm by ELISA with PPV coat-protein (CP) antiserum, tissue blot hybridization with PPV cRNA containing the viral CP gene, and RT-PCR using DNA primers for the CP. Differentiation from PPV by RT-PCR with primers for the 3' non-coding region (NCR) of the PPV genome
Détection d'un potyvirus chez du matériel génétique de pêcher chinois par ELISA à l'aide d'antisérum contre la protéine capside (CP) du PPV, par hybridation en 'tissue blot' avec un ARNc contenant le gène CP du virus, et par PCR avec des amorces ADN correspondant à la CP. Différenciation du PPV en RT-PCR avec amorces pour la zone 3' non codante du génome du PPV

Cultivar	Direct ELISA	Tissue blot	RT-PCR	
	Leaf tissue[a]	Leaf petiole	CP	NCR
Peach (Ta Tao #25)	1.677	+	+[b]	–[b]
N. clevelandii Infected with the virus from cv. Tao Tao 25	1.435	ND	+	–
Peach (Ku Chang Hung #14)	1.821	+	+	–
Peach (Ta Tao)	1.852	+	+	–
Peach-uninfected control	0.102	–	–	–
Peach (GF305) PPV-infected control	3.363	+	+	+
Plum, PPV-infected control	ND[b]	ND	+	+
Transgenic plum PRSV-CP control	ND	ND	+	–

[a] Samples consisted of tissues just above petioles of batched leaves. A_{405} values are the average of six wells.
[b] + positive result; – negative result; ND not determined.

Table 1 shows a comparison of the separate synthesis of the 220 bp DNA generated with primers for the 3' non-coding region of PPV with that of the 474 bp DNA fragment generated with PPV coat-protein primers using leaf nucleic acid extracts from the Chinese peach cultivars. RT-PCR products were amplified only when PPV coat-protein primers were used (Table 1). Thus, peach cultivars do not appear to be infected with PPV but rather by another previously undescribed virus, which has also been identified in *P. mume* cultivars imported by USA from Japan, several accessions of *P. mume* imported by Canada from Japan, and in several collections of peach cultivars of Chinese origin (results not shown). Our conclusions were substantiated by the facts that in RT-PCR assays: (i) the nucleic acid extracts from PPV-infected peach and plum controls reacted positively with two sets of differential primers, (ii) transgenic plums transformed with the coat-protein gene of papaya ringspot potyvirus (PRSV) (Scorza *et al.*, 1993) reacted positively only when DNA primers for PPV coat-protein gene were used, and (iii) uninfected plant controls reacted negatively with both sets of primers. Moreover, RT-PCR assays using PPV-specific DNA primers for the 3' non-coding region of PPV were positive for several PPV isolates from UK, Hungary, Romania, Czech Republic, Germany, Spain, Italy, Greece and Egypt and were negative for samples of several other potyviruses, including PVY and TEV (Levy & Hadidi, 1994).

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T-PCR with primers for the 3' non-coding region

pêcher chinois par ELISA à l'aide d'antisérum
n 'tissue blot' avec un ARNc contenant le gène CP
ant à la CP. Différenciation du PPV en RT-PCR
1 PPV

Tissue blot	RT-PCR	
Leaf petiole	CP	NCR
+	+[b]	−[b]
ND	+	−
+	+	−
+	+	−
−	−	−
+	+	+
ND	+	+
ND	+	−

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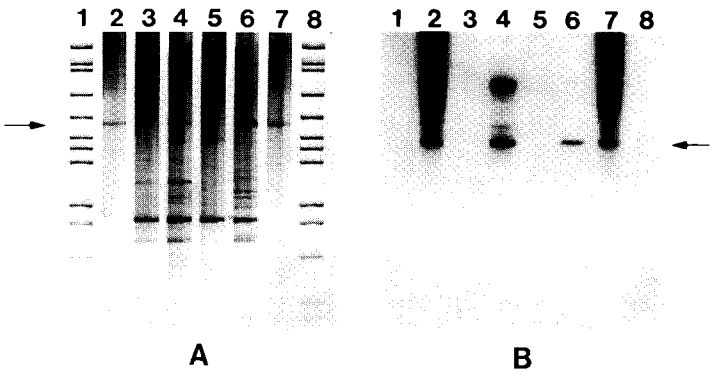


Fig. 1. Polyacrylamide gel electrophoretic analysis (A) and autoradiograph of Southern blot hybridization analysis (B) of cDNA amplification of segments of cloned PPV cDNA and of PPV and PVY genomes from total nucleic acids of infected tissue by primers for PPV coat-protein region. Amplified products of: cloned PPV cDNA (lanes 2 and 7), PPV-infected tobacco tissue (lane 4), PVY-infected potato tissue (lane 6), and uninfected tobacco or potato tissue control (lanes 3 and 5 respectively). Lane 1 shows pGEM DNA size marker, the arrow indicates 474 bp. Southern blot was hybridized with ^{32}P-labeled PPV cRNA probe containing PPV coat protein gene.
Analyse électrophorétique sur gel polyacrylamide (A) et autoradiographie en hybridation Southern blot (B) d'ADNc amplifié à l'aide d'amorces correspondant à la protéine capside du PPV, et provenant de segments d'ADNc cloné de PPV, ou d'acides nucléiques totaux des génomes du PPV ou du PVY extraits de tissus infectés. Produits amplifiés de: ADNc cloné de PPV (voies 2 and 7), tissus de tabac contaminés par le PPV (voie 4), tissus de pomme de terre contaminés par le PVY (voie 6), et tissus sains de tabac et de pomme de terre (voies 3 et 5 respectivement). La voie 1 correspond au marqueur ADN pGEM, la flèche indiquant la molécule à 474 paires de bases. En Southern blot, l'hybridation a été réalisée avec une sonde ARNc PPV marquée au ^{32}P et portant le gène de la protéine capside du PPV.

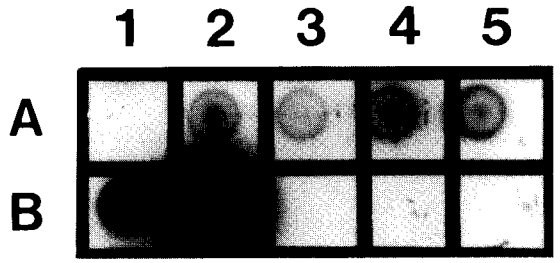


Fig. 2. Autoradiograph of dot-blot hybridization analysis of cDNA amplification of a segment of viral genome from nucleic acid extracts of east Asian *Prunus* germplasm by primers for PPV coat-protein region. Amplified products of east Asian *Prunus mume* (A, 1–3), east Asian peach (A, 4 and 5), PPV-infected plum and peach controls (B, 1 and 2), uninfected peach control (B, 3). Dot blot was hybridized with ^{32}P-labeled PPV cRNA probe containing PPV coat protein gene.
Autoradiographie en hybridation dot-blot d'un ADNc amplifié, à l'aide d'amorces correspondant à la protéine capside du PPV, à partir d'un segment de génome viral dans un extrait d'acides nucléiques de matériel génétique de *Prunus* d'origine est-asiatique. Produits amplifiés à partir de *P. mume* est-asiatique (A, 1–3), de pêcher est-asiatique (A, 4 et 5), de prunier et pêcher témoins infectés par le PPV (B, 1 et 2), et de pêcher sain (B, 3). L'hybridation dot-blot été réalisée avec une sonde ARNc PPV marquée au ^{32}P et portant le gène de la protéine capside du PPV.

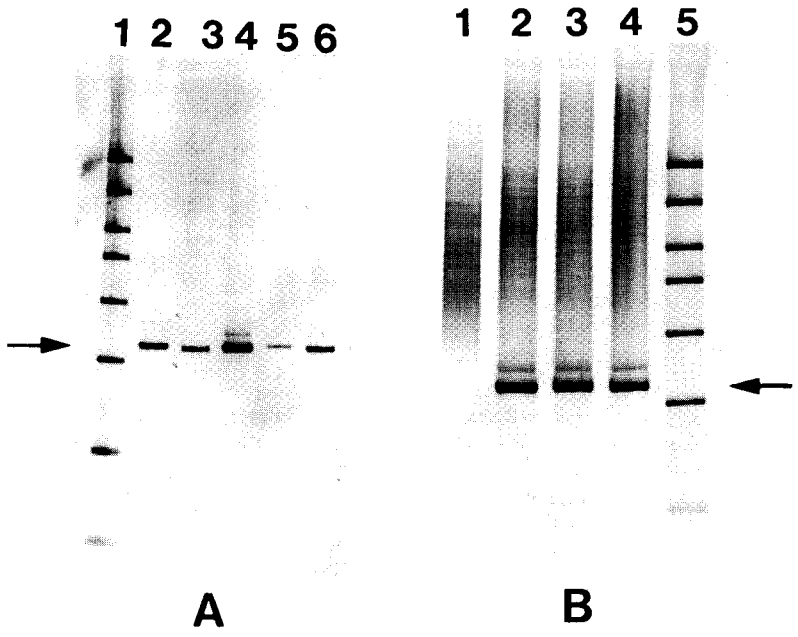
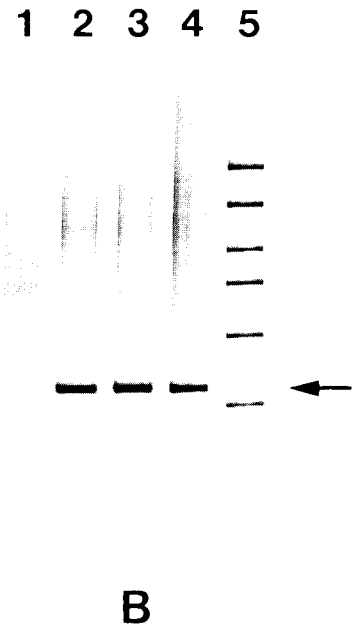


Fig. 3. Polyacrylamide gel electrophoretic analysis of PCR products of cloned PPV cDNA and of known PPV isolates from infected stone fruits (A) and from *Nicotiana benthamiana* (B). DNA primers for PPV 3' non-coding region were used for cDNA synthesis and amplification. BioLow DNA molecular weight marker with fragments size of (bp) 1000, 700, 500, 400, 300, 200, 100, 50 (A, lane 1 and B, lane 5), the arrow indicates 220 bp. (A) amplified products from: cloned PPV cDNA (lane 2); nucleic acid extracts from PPV-infected tissue from Spain (lane 3), isolate N_{23} on peach from Italy (lane 4), isolate A_3 on apricot from Italy (lane 5), and a plum isolate from Hungary (lane 6). (B) uninfected control (lane 1), PPV from the American Type Culture Collection (lane 2) and from UK (lanes 3 and 4).
Analyse électrophorétique sur gel polyacrylamide des produits PCR obtenus à partir d'ADNc cloné de PPV, ainsi que d'isolats connus de PPV sur fruits à noyau (A) ou sur *N. benthamiana* (B). Des amorces ADN pour la zone 3' non codante du PPV ont été utilisées pour la synthèse et l'amplification de l'ADNc. Marqueurs ADN BioLow avec fragments de (paires de bases) 1000, 700, 500, 400, 300, 200, 100, 50 (A, voie 1 et B, voie 5); la flèche indique 220 paires de bases. (A) produits amplifiés à partir de: ADNc cloné de PPV (voie 2); extraits d'acides nucléiques de tissus infectés par un PPV d'Espagne (voie 3), l'isolat pêcher N_{23} d'Italie (voie 4), l'isolat abricotier A_3 d'Italie (voie 5), et un isolat prunier de Hongrie (voie 6). (B) témoin sain (voie 1), PPV de l'American Type Culture Collection (voie 2) et du Royaume-Uni (voies 3 et 4).

The available complete nucleotide sequence data of three PPV strains (Laín *et al.*, 1989; Maiss *et al.*, 1989; Teycheney *et al.*, 1989), one strain each of PVY, TEV, and tobacco vein mottling potyvirus (Allison *et al.*, 1986; Domier *et al.*, 1986; Robaglia *et al.*, 1989), as well as 3' partial nucleotide sequence data for strains of PPV (Maiss *et al.*, 1989; Wetzel *et al.*, 1991; Cervera *et al.*, 1993), PVY, TEV, PRSV, watermelon mosaic 2 potyvirus, zucchini yellow mosaic potyvirus, Johnson grass mosaic potyvirus and sugarcane mosaic potyvirus (Allison *et al.*, 1985; Gough *et al.*, 1987; Rosner & Raccah, 1988; Frenkel *et al.*, 1989, 1991; Van der Vlugt *et al.*, 1989, 1993; Gal-on *et al.*, 1990; Grumet & Fang, 1990; Quemada *et al.*, 1990a, b) have provided valuable information regarding the high degree of sequence homology at the 3' non-coding region of strains of the same virus as opposed to those between distinct viruses. The 3' non-coding region differs considerably in nucleotide length and displays no sequence homology between distinct potyviruses, but high levels (from 83 to 99%) exist among isolates of the same



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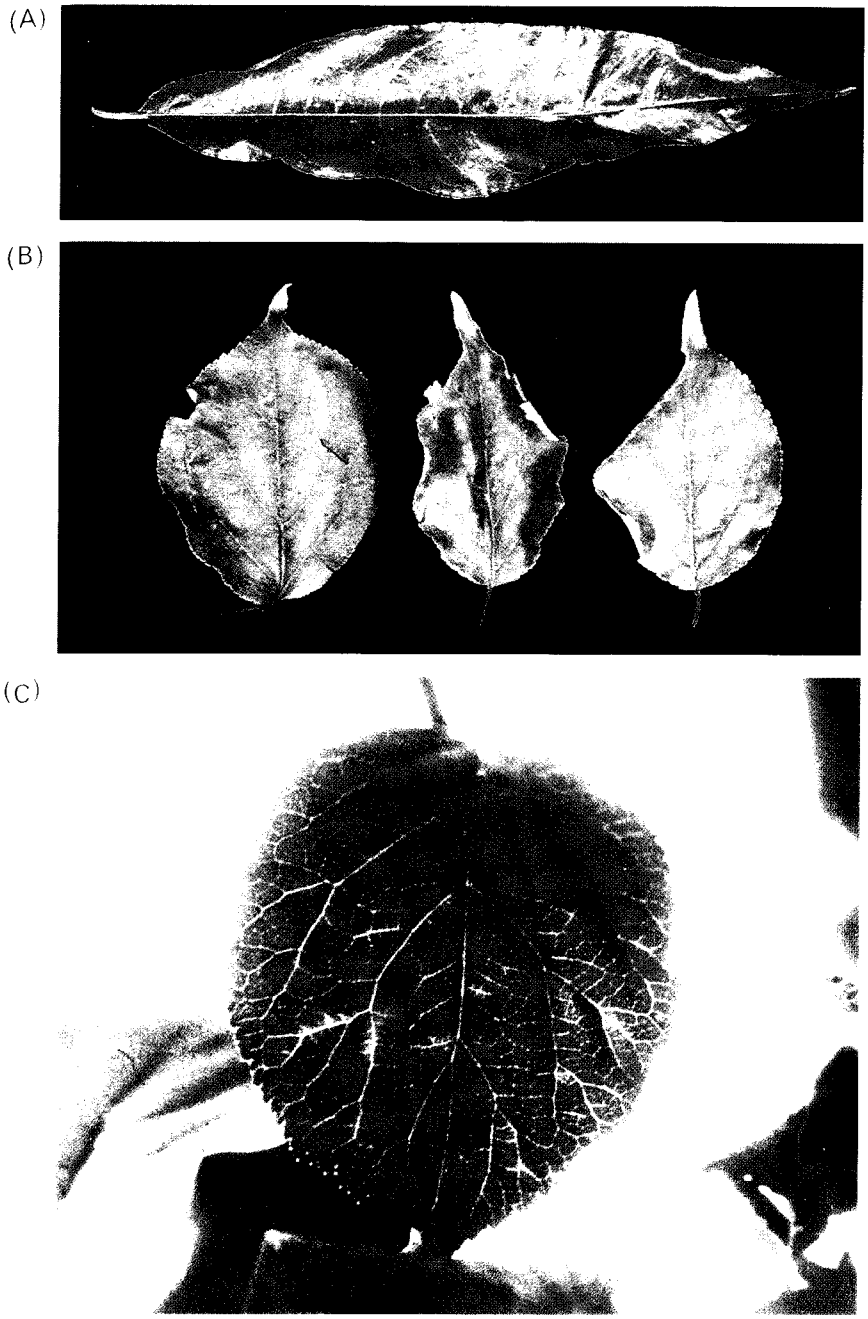
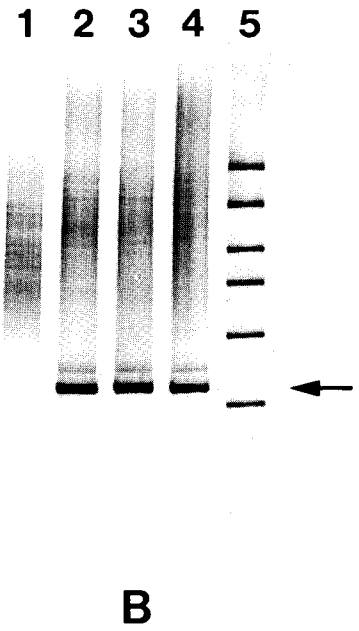


Fig. 4. Leaves of peach and *Prunus mume* infected with APLV. Symptomless APLV on peach cv. Ku Chung Hung (A) and on *P. mume* cv. Bungo (B) grown under screenhouse conditions. PPV-like symptoms on *P. mume* cv. Bungo may be induced by APLV for several days at 22 °C under controlled glasshouse conditions (C). Courtesy D. Thompson.

Feuilles de pêcher et de *P. mume* infectées par l'APLV. Absence de symptômes sur pêcher cv. Ku Chung Hung (A) et sur *P. mume* cv. Bungo (B) maintenus en screenhouse. Symptômes de type sharka sur *P. mume* cv. Bungo après plusieurs jours à 22 °C en serre climatisée (C). Photographie de D. Thompson.



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u (A) ou sur *N. benthamiana* (B). Des amorces
pour la synthèse et l'amplification de l'ADNc.
s) 1000, 700, 500, 400, 300, 200, 100, 50 (A, voie
duits amplifiés à partir de: ADNc cloné de PPV
un PPV d'Espagne (voie 3), l'isolat pêcher N_{23}
isolat prunier de Hongrie (voie 6). (B) témoin
(voie 2) et du Royaume-Uni (voies 3 et 4).

of three PPV strains (Lain *et al.*, 1989;
in each of PVY, TEV, and tobacco vein
., 1986; Robaglia *et al.*, 1989), as well as 3'
(Maiss *et al.*, 1989; Wetzel *et al.*, 1991;
lon mosaic 2 potyvirus, zucchini yellow
nd sugarcane mosaic potyvirus (Allison *et*
Frenkel *et al.*, 1989, 1991; Van der Vlugt
ng, 1990; Quemada *et al.*, 1990a, b) have
gree of sequence homology at the 3' non-
d to those between distinct viruses. The 3'
ength and displays no sequence homology
to 99%) exist among isolates of the same

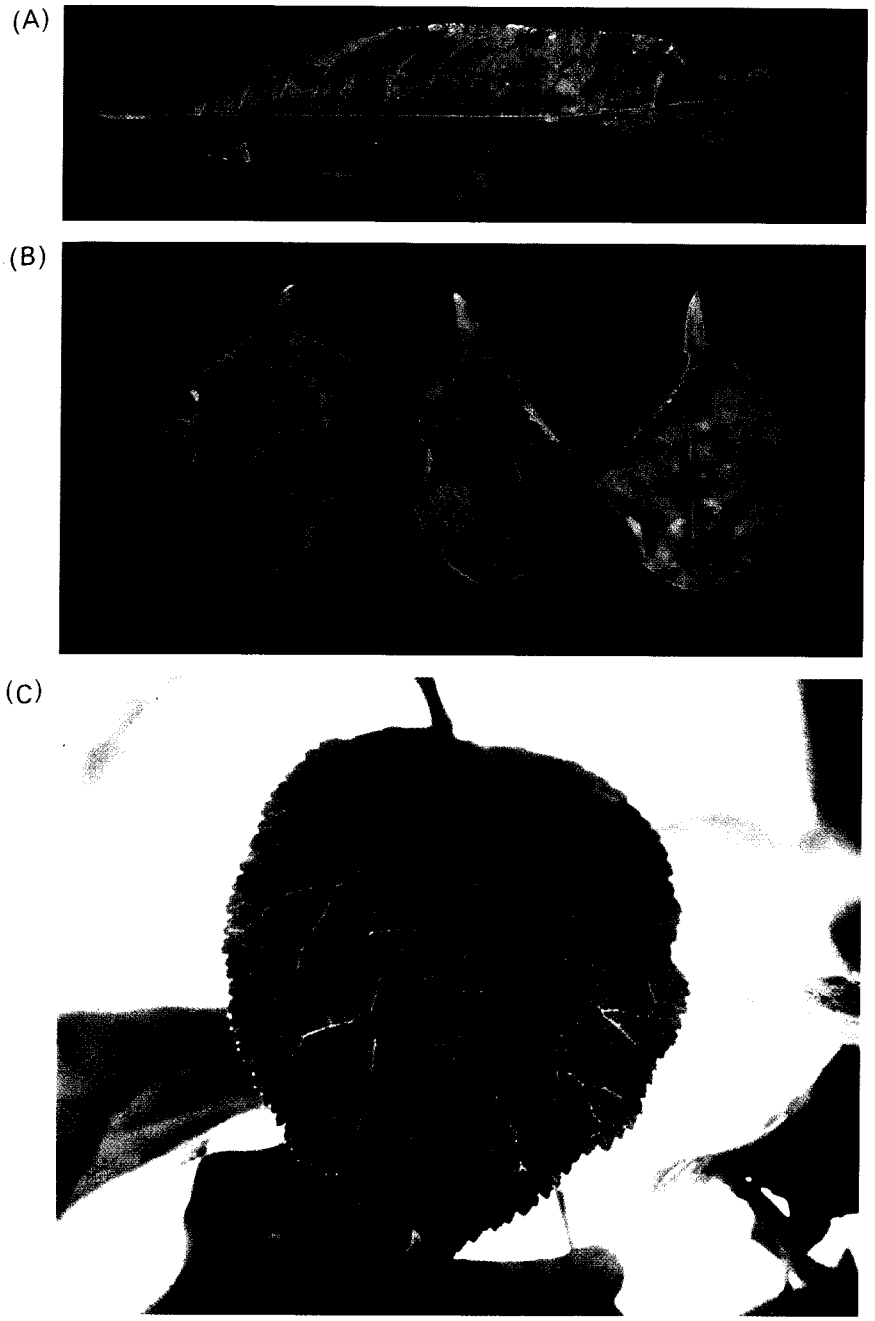

Fig. 4. Leaves of peach and *Prunus mume* infected with APLV. Symptomless APLV on peach cv. Ku Chung Hung (A) and on *P. mume* cv. Bungo (B) grown under screenhouse conditions. PPV-like symptoms on *P. mume* cv. Bungo may be induced by APLV for several days at 22 °C under controlled glasshouse conditions (C). Courtesy D. Thompson.

Feuilles de pêcher et de *P. mume* infectées par l'APLV. Absence de symptômes sur pêcher cv. Ku Chung Hung (A) et sur *P. mume* cv. Bungo (B) maintenus en screenhouse. Symptômes de type sharka sur *P. mume* cv. Bungo après plusieurs jours à 22 °C en serre climatisée (C). Photographie de D. Thompson.

virus (Frenkel *et al.*, 1989). Thus, the 3' untranslated sequence of PPV has great value in the specific differentiation of PPV from other potyviruses.

For these reasons, we suggest that the new virus from peach and *P. mume* that did not utilize the 3' non-coding region DNA primers of PPV in RT-PCR assays is not PPV. The positive reaction of PPV capsid-protein antiserum with the new virus suggests that it is a potyvirus, since capsid-protein antiserum of one potyvirus may react with variable numbers of potyviruses (Ward & Shukla, 1991). The new virus has, moreover, typical potyvirus particle morphology and is serologically related to PPV on the basis of the positive reaction with PPV capsid-protein antiserum in ELISA, and with CI antisera of tobacco etch and maize dwarf mosaic potyviruses in Western blot and ELISA analyses. We name the new virus prunus latent potyvirus because it does not cause visible symptoms on infected leaves of peach or *P. mume* grown under screenhouse conditions (Figs. 4A and B, respectively). PLV may, however, cause PPV-like symptoms (chlorotic vein banding) for several days in infected leaves of *P. mume* grown at 22 °C under controlled glasshouse conditions (Fig. 4C).

Our findings suggest that current PPV identification methods, which include the use of PPV coat-protein region for serological and molecular detection methods (Adams, 1978; Dosba *et al.*, 1986; Varveri *et al.*, 1987, 1988; Korschineck *et al.*, 1991; Levy & Hadidi, 1991; Wetzel *et al.*, 1992) may not differentiate between PPV and PLV. Screening for PPV using DNA primers and/or cDNA clones for the 3' non-coding region of PPV is to date PPV-specific and valuable in controlling PPV in *Prunus* domestically and during the international movement of germplasm.

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Identification précise du plum pox potyvirus permettant de le différencier de l'Asian prunus latent potyvirus sur du matériel génétique de *Prunus*

Nous avons déterminé, pour l'identification du plum pox potyvirus (PPV), la précision de la méthode RT-PCR, en utilisant des amorces d'ADN pour la zone 3' non codante du PPV comparativement à des amorces pour un segment correspondant à la protéine capside du PPV. Seules les amorces pour la zone 3' non codante ont permis d'identifier spécifiquement le PPV. Les amorces pour la protéine capside du PPV ont réagi avec d'autres potyvirus connus comme le potato Y potyvirus et le papaya ringspot potyvirus. La séquence de l'extrémité 3' non codante du PPV a donc une importance particulière, car elle permet de distinguer le PPV des autres potyvirus. D'après les connaissances actuelles, le PPV est le seul potyvirus signalé chez les *Prunus* spp. Or, nous venons d'identifier un virus latent sur du matériel génétique de pêcher et de *Prunus mume*, que nous dénommons l'Asian prunus latent potyvirus (APLV). Celui-ci peut être distingué du PPV par sa réaction en RT-PCR avec des amorces d'ADN de la région 3' non codante du PPV, mais réagit positivement en RT-PCR avec les amorces correspondant à la protéine capside du PPV, en hybridation moléculaire avec une sonde ADNc contenant le gène de la protéine capside du PPV, et en test sérologique avec des antisérums contre le PPV.

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Точная идентификация plum pox potyvirus и его отличие от Asian prunus latent potyvirus в селекционном материале Prunus

Установлена точность идентификации plum pox potyvirus (PPV) методом обратной транскрипции - полимеразной цепной реакции (RT-PCR) при использовании ДНК-праймеров для некодирующего 3'-концевого участка генома вируса PPV по сравнению с праймерами для фрагмента, соответствующего белку оболочки вируса PPV. Специфическими для идентификации вируса PPV оказались только праймеры для некодирующего 3'-концевого участка. Праймеры для белка оболочки вируса PPV реагировали с другими известными потивирусами, такими, например, как potato Y potyvirus и papaya ringspot potyvirus. Последовательность нуклеотидов 3'-концевого участка вируса PPV имеет большое значение в специфической дифференциации PPV от других потивирусов. PPV в настоящее время является единственным потивирусом, о которым имеются сведения, что он поражает *Prunus spp.* Идентифицирован латентный вирус, названный Asian prunus latent potyvirus (APLV), который инфицирует селекционный материал персика и *Prunus mume*. PPV может быть отличен от вируса APLV методом обратной транскрипции - полимеразной цепной реакции с использованием ДНК-праймеров, специфичных к некодирующей 3'-концевой области генома PPV. Однако APLV положительно реагирует с праймерами, соответствующими белку оболочки PPV, в методе обратной транскрипции - полимеразной цепной реакции, с зондом клонированной кДНК PPV, содержащей ген белка оболочки, в методе молекулярной гибридизации и с антисывороткой к PPV в серологических испытаниях.

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