A BROAD-SPECTRUM PCR ASSAY COMBINED WITH RFLP ANALYSIS FOR DETECTION AND DIFFERENTIATION OF PLUM POX VIRUS ISOLATES

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Abstract

Primers from the NIb (replicase) gene of plum pox virus (PPV) were used in a reverse transcription-polymerase chain reaction (RT-PCR) assay to detect field isolates from apricot and plum trees. A PPV-specific PCR product of c.1040bp was obtained from each infected tree. PCR products were digested with restriction enzymes, and restriction fragment length polymorphism (RFLP) patterns were compared to those from characterized isolates PPV-D, PPV-Ran, and PPV-NAT (D-serotype), PPV-SK68 (M-serotype), PPV-El Amar, and PPV-SoC (sour cherry). PCR products of characterized D-serotype isolates had a *Taq I* RFLP pattern that was distinct from patterns unique to each of SK68, El Amar, and SoC isolates. PPV-NAT was distinguished from PPV-D and PPV-Ran by *Dde I* and *Rsa I* RFLPs; SK68, El Amar, and SoC each had unique RFLP patterns with each enzyme. Field isolates could be differentiated by RFLP patterns; most had *Taq I* RFLPs typical of the D-serotype, but PCR products from some trees produced RFLP patterns distinct from any of the characterized isolates. Heterogeneity in the viral population from single trees was also observed, with two distinct RFLP patterns obtained from PCR products of some trees. The NIb PCR product is much larger than the coat protein (CP) PCR product of Wetzel *et al.*, (1991a; 1040bp compared to 243bp), and more restriction sites differentiate between and within serotypes. Thus SK68 (M-serotype) was distinct from El Amar, SoC and D-serotype isolates, and some D-serotype isolates were differentiated. The combination of the PPV NIb RT-PCR with RFLP analysis will be valuable for studies of epidemiology and phylogeny.

1. Introduction

PPV is economically the most important virus of stone fruit trees in Europe and the Mediterranean area. It causes reduced fruit quality, premature fruit drop, and rapid decline and death of trees also infected with other viruses (Nemeth, 1986). Eradication programs have had partial success (e.g. Dunez and Sutic, 1988), but PPV continues to spread throughout Europe. PPV has not been reported in North America, where it is a quarantine concern, but has already reached South America (Roy and Smith, 1994). Difficulties in indexing for PPV result from serological differences between isolates (Sutic, 1971; Kerlan and Dunez, 1979; Grüntzig and Fuchs, 1986), although broadly reactive or serotype-specific monoclonal antibodies are now available (e.g. Cambra *et al.*, 1994). Biological indexing continues to be used to detect PPV, but the widely used peach GF305 indicator host does not react differentially to the various serotypes. *Prunus tomentosa* reacts to PPV-M more severely than to D-serotype isolates, allowing serotype differentiation (Damsteegt *et al.* 1997). PPV serotypes correlate well with biological properties, with the M-serotype causing more severe disease than the D-serotype (Bousalem *et al.*, 1994; Candresse *et al.*, 1995; Damsteegt *et al.*, 1997).

Molecular techniques have also been used to detect PPV. Varveri *et al.* (1987, 1988) used PPV-D CP gene cDNA and cRNA probes to detect PPV with greater sensitivity than ELISA, and non-structural gene probes had greater cross-hybridization with M-serotype isolates

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(Wetzel *et al.*, 1990). Linked RT-PCR detection using PPV CP gene primers amplified multiple strains, and presence of an *Rsa I* site in the 243bp product distinguished D-serotype isolates from PPV-M and the divergent El Amar isolate (Wetzel *et al.*, 1991a). The sensitivity of RT-PCR was improved with an immunocapture step to concentrate PPV from a larger sample volume (Wetzel *et al.*, 1992). Deborré *et al.* (1995) used another CP gene primer pair to amplify a 451bp fragment, and describe *Dra I* and *Sfu I* polymorphisms that correlate with the previously described *Rsa I* polymorphism. Candresse *et al.* (1995) designed D- and M- serotype-specific primers, allowing serotype determination by PCR. Hadidi and Levy (1994) and Levy *et al.* (1995) have described a broad-spectrum PPV PCR assay based on the 3' non-coding region of PPV-D.

While the CP primers detect all isolates of PPV tested (Wetzel *et al.*, 1991a,1992; Candresse *et al.*, 1995; Krczal and Avenarius, 1995; Corvo *et al.*, 1995), the *Rsa I* polymorphism only distinguished D-serotype isolates from other serotypes. We have developed primers from the NIb (polymerase) gene, amplifying a 1040bp product that allows greater differentiation between and even within serotypes using multiple restriction enzymes. Using the combined RT-PCR/RFLP assay we have identified distinct RFLP patterns that differentiate PPV-D serotype isolates from PPV-M. The El Amar isolate and the sour cherry (PPV-SoC; Nemchinov and Hadidi, 1996) isolate that appear to represent separate serogroups (Candresse *et al.*, 1995; Nemchinov *et al.*, 1996) were also distinguished by distinct RFLP patterns. The ability to distinguish between strains within a serotype should be useful for detection of mixed infections, and for epidemiological studies.

2. Materials and methods

2.1. PCR primers

The sequences of the complete NIb genes of PPV-NAT (D13751; Maiss *et al.*, 1989), PPV-D (X16415; Teycheney *et al.*,), PPV-Ran (M21847; Lain *et al.*, 1988), PPV-SK68 (M92280; Palkovics *et al.*, 1993), PPV-El Amar (X56258; Wetzel *et al.*, 1991b) and partial NIb sequences of PPV-PS (S57405; Cervera *et al.*, 1993) and PPV-ō6 (S57404 ; Cervera *et al.*, 1993) were aligned with the aid of PC-GENE (Intelligenetics). Primers PPV-NIb-F (5' GCTGTIGATGTIGTCATITCIATG 3'; corresponding to PPV-NAT nt7299-7322) and PPV-NIb-R (ĀCCATTĠATGCĀCĀAATTGCCTC; the complement of PPV-NAT nt8316-8338) were selected; four inosine residues (underlined) were substituted in PPV-NIb-F to allow for differences between the sequences.

2.2. Plasmids and virus isolates

Plasmids with cDNA inserts containing the NIb genes of PPV-NAT and PPV-Ran (D serotype), PPV-SK68 (M serotype) and PPV-El Amar were generous gifts of E. Maiss, J.A. Garcia, L. Palkovics and T. Candresse respectively. A *Bgl II/Kpn I* restriction fragment including the PPV-D NIb gene was a gift of M. Ravelonandro. Purified RNA of PPV-SoC was a gift of L. Nemchinov and A. Hadidi. Field isolates of PPV from naturally infected apricot trees were obtained from the research orchard of the University of Agriculture Institute of Pomology, Gerasdorf; naturally infected plum samples were from a private orchard in Poysdorf, Austria. Samples included both symptomatic and symptomless leaves, and additionally bark samples from some symptomatic trees.

2.3. Sample preparation and reverse transcription

Total RNA extracts from leaf or bark samples were prepared according to Chang *et al.* (1993), from either 3g or 0.5g of tissue. RNA (0.2-1µg in sterile distilled water) was mixed with 0.5µg primer PPV-NIb-R, incubated at 70°C for 5 min., and allowed to cool to room temperature before addition of buffer, nucleotides and 12 units of AMV reverse transcriptase

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3. Results

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(Boehringer) to bring the final volume to 25µl. The reverse transcription reaction was incubated for 1 h at 42°C.

2.4. PCR reactions and analysis

For initial amplifications, 5µl of the reverse transcription mix or 2µl of appropriately diluted plasmid was amplified in a 50µl reaction containing 10pmol each of primers PPV-NIb-F and PPV-NIb-R, 1 unit *Taq* polymerase, 3mM $MgCl_2$, 25mM KCl, 0.05% Tween 20, 100µg/ml BSA, 50µM each dNTP, and 20mM Tris-HCl pH 8.3. Amplification was for one cycle at 94°C for 3 min, 40 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, followed by one cycle of 72°C for 5 min. A 15µl sample was analyzed on a 5% acrylamide gel, and stained with ethidium bromide. For further amplification the viral-specific product was excised from the gel and purified by crushing the gel and soaking overnight in 200µl of 0.5M ammonium acetate, 1mM EDTA, prior to centrifugation to pellet gel fragments. The supernatant was transferred to a fresh microfuge tube and DNA precipitated with isopropanol; each gel-purified PCR product was then resuspended in 50µl sterile water. An aliquot was diluted 1:20 with sterile water, and 2µl subjected to PCR with cycle times reduced to 94°C for 45s, 60°C for 45s, and 72°C for 90s. Plasmid DNA from characterized PPV isolates was similarly amplified.

2.5. RFLP analysis

PCR products were ethanol precipitated and resuspended in sterile water. Aliquots were then digested with *Dde I*, *Rsa I*, or *Taq I*, and the fragments analyzed on acrylamide gels, and visualized with ethidium bromide.

3. Results

3.1. PCR from plasmid DNA and total RNA

A single fragment of about the expected size (1040bp) was amplified from each of the cloned, characterized PPV isolates; the PCR products from PPV-SK68 and PPV-El Amar isolates consistently migrated slightly faster than the products from other isolates (Figure 1A). The PCR products of PPV-D and PPV-SoC were cloned to provide standards for further use. A more complex pattern of products was obtained from initial amplification of total RNA extracts of plant tissue. The specific c.1040bp fragment was visible from all reactions, including samples from trees without apparent symptoms; varying numbers of smaller products were present in different samples (Figure 2). Samples of 0.5g yielded similar products to 3g samples from the same trees (data not shown). There was lower intensity of bands from non-symptomatic compared to symptomatic tissues from trees of the same variety, but the amplification product was clearly visible (e.g. Figure 2; samples 7A and 8A symptomatic, and 9A non-symptomatic apricot Marille Schaar). To allow further analysis of the PPV-specific fragment, the c.1040bp bands from field-infected samples were gel purified and re-amplified, typically yielding in a major product of c.1040bp and few minor products (e.g. Figure 1A).

3.2. RFLP analysis of PCR products

PPV-specific products from naturally-infected trees were compared to those from cloned PPV isolates following digestion with *Dde I*, *Rsa I*, or *Taq I*. Differences between isolates were observed with each restriction enzyme. In each case the RFLP patterns of PPV-SK68 (M-serotype), -El Amar, and -SoC were distinct from each other, and also from the PPV-D, -Ran, and -NAT isolates (D-serotype) (Figure 1B-D). More restriction fragments differed between SK68 and D-serotype isolates with each enzyme than were common to both digests (Figure 1B-D). PPV-NAT was distinguished from PPV-D and PPV-Ran by *Dde I* and *Rsa I*,

but not *Taq I* RFLP patterns (Figure 1B-D). Most of the apricot and plum samples yielded RFLP patterns equivalent to PPV-D/PPV-Ran with *Dde I* and *Taq I*. However, several samples yielded an *Rsa I* RFLP pattern distinct from any of the cloned characterized isolates, and additional novel *Dde I* and *Taq I* RFLP patterns were also observed in some samples (e.g. apricot #1, apricot #6; Figure 1B-D). Several samples yielded complex patterns that appeared to result from the presence of at least two distinct sequences, as the sum of the restriction fragments was greater than 1040bp, and some fragments corresponded to RFLP patterns observed in other samples (e.g. apricot #4,apricot #11; Figure 1B-D).

4. Discussion

Primers designed from conserved sequences in the NIb (replicase) gene of characterized PPV isolates were shown to yield an amplified product of the expected size from all field-infected trees tested and cloned cDNAs. Non-specific products from total RNA might be reduced by using immunocapture RT-PCR. While PCR products from PPV-SK68 and PPV-El Amar consistently migrated faster than those of the characterized D-serotype isolates and products from the majority of field samples, the products predicted from the published sequences are identical at 1040bp. The difference in migration may be due to sequence-specific DNA conformation. Observed restriction fragment sizes for PCR products of characterized isolates correlated well with those predicted from the published sequences, although small (<50bp) predicted fragments were not retained in the gel or stained too weakly to be visible (data not shown). Additional enzymes are predicted to differentiate between or within serotypes (data not shown).

The major difference in *Dde I* RFLP patterns results from only a single *Dde I* site being conserved between D-serotype isolates and M-serotype SK68. Within characterized D-serotypes, PPV-NAT lacks two *Dde I* sites and one *Rsa I* site present in PPV-D and PPV-Ran (data not shown), yielding distinct RFLP patterns. Whereas characterized D-serotype isolates have a common *Taq I* RFLP profile, only two of nine *Taq I* sites present in PPV-SK68, and no restriction fragments, are conserved with D-serotype isolates. RFLP patterns of SK68, El Amar and SoC were distinct from each other (Figure 1B-D). Only two *Dde I* (and a 46bp fragment), two *Taq I* sites (and a 5bp fragment), and one *Rsa I* site (and a 100bp fragment) are common to the SK68 and El Amar sequences. These isolates and PPV-SoC are thus distinct, as shown by sequence analysis and serology (Candresse *et al.*, 1995; Nemchinov *et al.*, 1996). The RFLP patterns of PPV-El Amar may thus be regarded as the prototype of the "E" serotype, and those of PPV-SoC of the "C" serotype.

RFLP patterns ofapricot and plum field isolates were mainly typical of characterized D-serotype isolates; none were similar to SK68, El Amar or SoC (Figure 1B-D; and data not shown). However, PCR products of some isolates yielded RFLP patterns distinct from any characterized isolate; for example,apricot #1,apricot #6 andapricot #10 produced distinct patterns with each of the enzymes tested (Figure 1B-D). Based on what is known of serotype distribution, it is probable that these are D-serotype isolates.

Some samples had complex RFLP patterns that are suggested mixed infections; examples areapricot #4 andapricot #11 (Figure 1B-D). There were differences in staining intensity of distinct bands, and fragment sizes totalled more than 1040bp. Natural mixed infections of serologically distinct PPV isolates have been reported previously (e.g. Asensio *et al.*, 1995; Pasquini *et al.*, 1995). Similar detection of mixed potyvirus isolates by RFLP analysis of PCR products has been reported by Langeveld *et al.* (1991).

We have cloned the PCR products from some samples with distinctive RFLP patterns, and from samples with apparent mixtures of isolates, in order to sequence the amplified portion of these isolates and determine how closely the individual isolates are related to the well characterized isolates. Similar analysis of CP sequences by Candresse *et al.* (1995) provided evidence for the separation of PPV-El Amar from M-serotype isolates.

The primers from the NIb gene were shown to amplify all characterized PPV serotypes and field isolates tested, and are expected to amplify almost any isolate of PPV. RFLP analysis showed not only that serotypes could be distinguished from characteristic

polymorphisms with multiple enzymes, but that isolates within a serotype could be differentiated. This assay has the advantage over the CP PCR and *Rsa I* polymorphism assay (Wetzel *et al.*, 1991a), and serotype-specific primers (Candresse *et al.*, 1995), that a single PCR reaction can be used to both detect all isolates, and by RFLP analysis of the large PCR product, to differentiate between many isolates. This should be of considerable value in epidemiological and phylogenetic studies.

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References

Asensio M., Gorris M.T., Sanz A., Camarasa E., Pérez E., Carbonell E.A., and Cambra M., 1995. Acta Horticulturae 386: 354-356.

Bousalem M., Candresse T.,Quiot-Douine L., and Quiot J.B. 1994. Comparison of three methods for assessing plum pox virus variability: further evidence for the existence of two major groups of isolates. J. Phytopathol. 142: 163-172.

Cambra M., Asensio M., Gorris M.T., Pérez E., Camarasa E., Garcia J.A., Moya J.J., López-Abella D., Vela C., and Sanz A., 1994. Detection of plum pox potyvirus using monoclonal antibodies to structural and non-structural proteins. EPPO Bulletin 24: 569-577.

Candresse T., MacQuaire G., Lanneau M., Bousalem M., Quiot-Douine L., Quiot J.B., and Dunez J., 1995. Analysis of plum pox virus variability and development of strain-specific PCR assays. Acta Horticulturae 386: 357-369.

Cervera M.T., Riechmann J.L., Martin M.T., and Garcia J.A., 1993. 3'-terminal sequence of the plum pox virus PS and ŏ6 isolates: evidence for RNA recombination within the potyvirus group. J. Gen. Virol. 74: 329-334.

Chang S., Puryear J., and Cairney J., 1993. A simple and efficient method for isolating RNA from pine trees. Plant Mol. Biol. Reptr. 11: 113-116.

Corvo, L.M., Sousa Santos, M., and Nolasco, G. 1995, Detection of plum pox virus using a simplified polymerase chain reaction procedure. Acta Horticulturae 386: 383-390.

Damsteegt V.D., Waterworth H.E., Mink G.I., Howell W.E., and Levy L., 1997. *Prunus tomentosa* as a diagnostic host for detection of plum pox virus and other *Prunus* viruses. Plant Dis. 81: 329-332.

Deborré G., Maiss E., and Jelkmann W., 1995. Biological and molecular biological investigations of several plum pox virus (PPV) isolates. Acta Horticulturae 386: 253-262.

Dunez J. and Sutic D., 1988. Plum pox. In: European Handbook of Plant Diseases. I.M. Smith *et al.*, eds. Blackwell Scientific, Oxford, U.K. Pp. 44-46.

Grüntzig M. and Fuchs E., 1986. Untersuchungen zur Differenzierung von Stämmen des Scharka-Virus (plum pox virus, PPV). Z. Pflanzenkrank. Pflanzenschutz. 93: 19-23.

Hadidi A. and Levy L., 1994. Accurate identification of plum pox virus and its differentiation from asian prunus latent potyvirus in *Prunus* germplasm. EPPO Bulletin 24: 633-643.

Kerlan C. and Dunez J., 1979. Différenciation biologique et sérologique de souches du virus de la sharka. Ann. Phytopathol. 11: 241-250.

Krczal G. and Avenarius U., 1995. Detection and characterization of plum pox virus (PPV) isolates from southern Germany by polymerase chain reaction (PCR). Acta Horticulturae 386: 370- 375.

Lain S. Riechmann J.L. Méndez E., and Garcia J.A., 1988. Nucleotide sequence of the 3' terminal region of plum pox potyvirus RNA. Virus Res. 10: 325-342.

Langeveld S.A., Dore J-M., Memelink J., Derks A.F.L.M.,Van der Vlugt C.I.M., Asjes C.J., and Bol J.F., 1991. Identification of potyviruses using the polymerase chain reaction with degenerate primers. J. Gen. Virology 72: 1531-1541.

Levy L., Kolber M., Tokes G., Nemeth M., and Hadidi A., 1995. 3' non-coding region RT-PCR detection and molecular hybridization of plum pox virus in anthers of infected stone fruit. Acta Horticulturae 386: 331-339.

Maiss E., Timpe U., Brisske A., Jelkmann W., Casper R., Himmler G., Mattanovich D., and Katinger H.W.D., 1989. The complete nucleotide sequence of plum pox virus RNA. J. Gen. Virology 70: 513-524.

Nemchinov L. and Hadidi A., 1996. Characterization of the sour cherry strain of plum pox virus. Phytopathol. 86: 575-580.

Nemchinov L., Hadidi A., Maiss E., Cambra M., Candresse T., and Damsteegt V., 1996. Sour cherry strain of plum pox potyvirus (PPV): molecular and serological evidence for a new subgroup of PPV strains. Phytopathol. 86: 1215-1221.

Nemeth M., 1986. Virus, Mycoplasma, and Rickettsia Diseases of Fruit Trees. Martinus Nijhoff Publ.

Palkovics L., Burgyán J., and Balázs E., 1993. Comparative sequence analysis of four complete primary structures of plum pox virus strains. Virus Genes 7: 339-347.

Pasquini G., Mazzei M., Pilotti M., and Barba M., 1995. Characterization of PPV isolates using monoclonal antibodies. Acta Horticulturae 386: 346-353.

Roy A.S. and Smith I.M., 1994. Plum pox situation in Europe. EPPO Bulletin 24: 515-523.

Sutic D., 1971. Etat des récherches sur le virus de la sharka. Ann. Phytopathol. INRA Publ.71: (2) 161.

Teycheney P.Y., Tavert G., Delbos R., Ravelonandro M., and Dunez J. 1989. The complete nucleotide sequence of plum pox virus RNA (strain D). Nucleic Acids Res. 17: 10115-10116.

Varveri C., Ravelonandro M., and Dunez J., 1987. Construction and used of a cloned cDNA probe for the detection of plum pox virus in plants. Phytopathol. 77: 1221-1224.

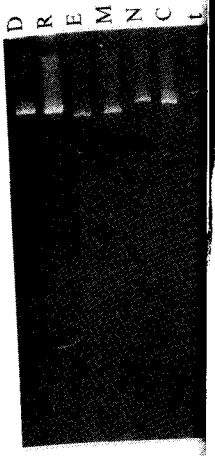
Varveri C., Candresse T., Cugusi M., Ravelonandro M., and Dunez J., 1988. Use of a ^{32}P-labeled transcribed RNA probe for dot hybridization detection of plum pox virus. Phytopathol. 78: 1280-1283.

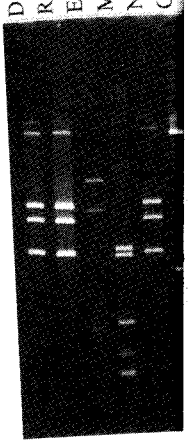
Wetzel T., Tavert G., Teycheney P.Y., Ravelonandro M., Candresse T., and Dunez J., 1990. Dot hybridization detection of plum pox virus using ^{32}P-labeled RNA probes representing non-structural viral protein genes. J. Virol. Methods 30: 161-172.

Wetzel T., Candresse T., Ravelonandro M., and Dunez J., 1991a. A polymerase chain reaction assay adapted to plum pox potyvirus detection. J. Virol. Methods 33: 355-365.

Wetzel T., Candresse T., Ravelonandro M., Delbos R.P., Mazyad H., Aboul-Ata, A.E., and Dunez J., 1991b. Nucleotide sequence of the 3'-terminal region of the RNA of the El-Amar strain of plum pox potyvirus. J. Gen. Virology 72: 1741-1746.

Wetzel T., Candresse T., Macquaire,G., Ravelonandro M., and Dunez J., 1992. A highly sensitive immunocapture polymerase chain reaction method for plum pox potyvirus detection. J. Virol. Methods 39: 27-37.





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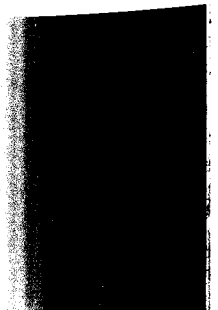

n der Vlugt C.I.M., Asjes C.J.,
polymerase chain reaction with

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mmler G., Mattanovich D., and
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sour cherry strain of plum pox

e T., and Damsteegt V., 1996.
r and serological evidence for a

eases of Fruit Trees. Martinus

ive sequence analysis of four
us Genes 7: 339-347.
haracterization of PPV isolates
-353.
. EPPO Bulletin 24: 515-523.
arka. Ann. Phytopathol. INRA

l Dunez J. 1989. The complete
Nucleic Acids Res. 17: 10115-

on and used of a cloned cDNA
athol. 77: 1221-1224.
Dunez J., 1988. Use of a ^{32}P-
detection of plum pox virus.

ndresse T., and Dunez J., 1990.
beled RNA probes representing
1-172.
., 1991a. A polymerase chain
Virol. Methods 33: 355-365.
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and Dunez J., 1992. A highly
ethod for plum pox potyvirus

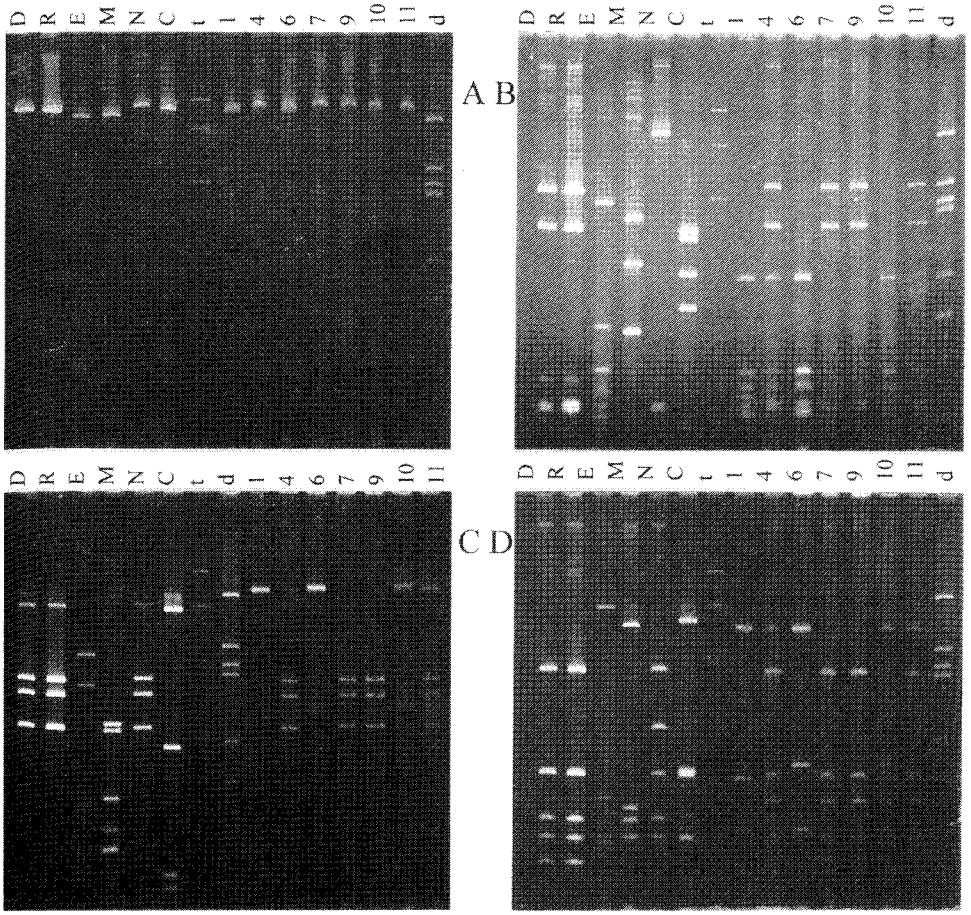

Figure 1 - Gel analysis of PCR products from cloned PPV cDNA and re-amplified reverse-transcribed RNA. A: undigested; or digested with B: *Dde I*; C: *Taq I*, and D: *Rsa I*. Samples in each case are: D, PPV-D; R, PPV-Ran; E, PPV-El Amar; M, PPV-SK-68; N, PPV-NAT; C, PPV-SoC; 1, apricot #1; 4, apricot #4; 6, apricot #6; 7, apricot #7; 9, apricot #9; 10, apricot #10; 11,apricot #11; d and t, DNA size standards (pUC19 digested with *Dde I* and *Taq I* respectively).

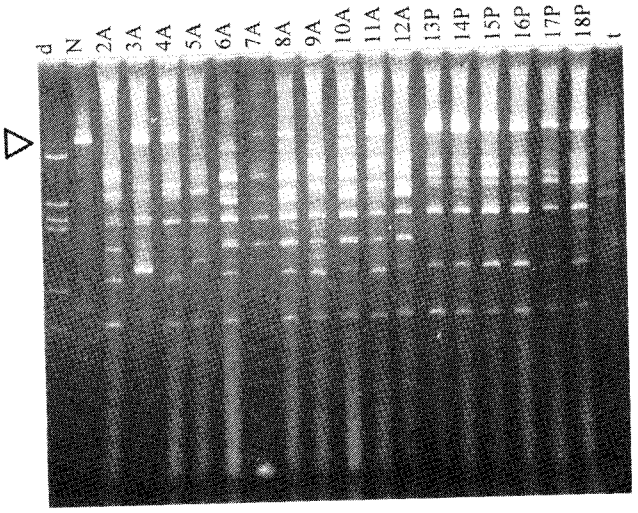

Figure 2 - Gel analysis of PCR products from initial amplification of reverse-transcribed RNA fromapricot (lanes 2-12; samples apricot 2A-12A) and plum (lanes 13-18; plum 13P-18P) trees. N, PPV-NAT control; d and t, DNA size standards (pUC19 cut with *Dde I* and *Taq I* respectively). Note the PPV-specific product (arrow) visible in each sample.

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Abstract

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