Etiology

Characterization of the Sour Cherry Strain of Plum Pox Virus

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ABSTRACT

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Properties of the sour cherry isolate of plum pox virus (PPV) were investigated by reverse transcription-polymerase chain reaction (RT-PCR), restriction fragment length polymorphism (RFLP), molecular hybridization, nucleotide sequencing, ultrathin sectioning of infected tissue, and graft transmission to different cherry rootstocks. Analysis of RT-PCR–amplified cDNA product from infected tissue with primers for the 3′ noncoding region (3′-NCR) of the PPV genome and molecular hybridization of the amplified product with a labeled PPV cRNA probe verified that the potyvirus infecting sour cherry trees (*Prunus cerasus*) in Moldova is an isolate of PPV. RFLP analysis of RT-PCR products from infected tissue with specific primers for the 3′-terminal region of the PPV coat protein (CP) gene revealed that the sour cherry isolate of PPV is a unique strain of PPV and a prototype of a new group that contains neither the *Rsa*I nor the *Alu*I restriction site. These results were confirmed by nucleotide sequencing analysis. Nucleotide sequencing of the 3′-NCR and the region coding for the 3′-terminal fragment of the PPV CP gene showed about 93% identity to that of other PPV isolates. RT-PCR assays of tissue extracts from three sour cherry cultivars demonstrated that sour cherry PPV was distributed systemically in sour cherry trees and infected leaf, bark, root, flower, fruit, and seed tissues. The virus was successfully transmitted by chip bud grafting to rootstocks of *P. avium* (sweet cherry) and *P. mahaleb*.

Additional keywords: detection, quarantine regulations.

Plum pox disease (sharka) is caused by plum pox potyvirus (PPV) (7). Sharka is the most important viral disease of stone fruit trees in Europe and the Mediterranean region because of reduced fruit quality, premature fruit drop, rapid natural spread by aphid vectors, and rapid decline and death of trees when coinfected with other viruses (25). Among stone fruits, cherry trees have been considered resistant to PPV infection (6,25). Experimental transmission of several isolates of PPV (from plum, peach, and apricot) to *Prunus avium* and *P. mahaleb* showed that graft-inoculated plants were negative in enzyme-linked immunosorbent assay (ELISA) for PPV, but plants inoculated through aphid transmission were ELISA positive (6). PPV remained localized to the infection site and became undetectable.

Recent reports from Moldova have claimed that sour cherry (*Prunus cerasus* L.) is naturally infected with PPV, as shown by biological and serological assays (11,12). The sour cherry isolate of PPV (PPV-SoC) differs from conventional PPV isolates that affect plum, apricot, and peach in Moldova by its transmission to a wide range of herbaceous hosts and its symptomatology (12). ELISA and immunosorbent electron microscopy (ISEM) do not differentiate between sour cherry and plum isolates of PPV (12).

We utilized PPV-specific primers in reverse transcription-polymerase chain reaction (RT-PCR) assay in combination with restriction fragment length polymorphism (RFLP), molecular hybridization, nucleotide sequencing, ultrasectioning of infected tissue, and viral transmission by chip budding to characterize the potyvirus affecting sour cherry trees in Moldova. In this paper, we show that the agent is PPV and that it is a prototype of a new PPV group. We also demonstrate that PPV-SoC is distributed systemically in infected sour cherry (*P. cerasus*) trees and is transmitted by chip budding from *P. cerasus* to *P. mahaleb* and sweet cherry (*P. avium*). A preliminary account of these results has been reported (21, 23,24).

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MATERIALS AND METHODS

Plant materials. All plant samples used in this study were collected from the sour cherry orchard of the Horticultural Research Institute, Kishinev, Moldova. Sour cherry cultivars Orlovskaya Konservnaya, Pandy, and Hybrid 8-34-3 naturally infected with PPV were used in this study. The infected trees showed characteristic chlorotic ringspot symptoms on leaves and depressions, necrosis, and rings on fruit. Collected tissues tested positive for PPV and negative for Prunus necrotic ringspot and prune dwarf ilaviruses and apple chlorotic leaf spot trichovirus by ELISA and ISEM. Tissues collected from cultivars Zshedraya and uninfected Pandy that tested negative for PPV by ELISA and ISEM were used as uninfected controls. PPV-infected plum, apricot, and peach samples also were obtained from the Horticultural Research Institute in Kishinev.

Sample preparation for PCR. Two pairs of PCR primers were utilized in this study. The first pair was specific for the 3′ noncoding region (3′-NCR) of PPV RNA (9,15), and the second pair was designed for the 3′-terminal region of the PPV coat protein (CP) gene (28). Two methods were used to prepare samples for RT-PCR: sample extraction utilizing Gene Releaser (GR; BioVentures, Murfreesboro, TN) (15,17) or immunocapture (IC) of virions (1,8,19,22,29) with an antiserum to PPV (29).

Crude plant extracts were prepared by grinding each fresh leaf sample (0.5 to 1.0 cm^2) or 50 to 100 mg of seed tissue or dried leaf, bark, root, or flower tissue with disposable pestles (Kontes, Vineland, NJ) in sterilized microcentrifuge tubes containing 100

μl of ice-cold TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and 0.2 to 0.4 mg of 120-grit Carborundum per milliliter. Samples were centrifuged at 14,000 rpm for 2 to 5 min at 4°C in an Eppendorf centrifuge. Aliquots (1 to 2 μl) of supernatant were added to 23 μl of GR polymeric matrix in a sterile 0.5-ml microcentrifuge tube. The mixture was vortexed for 30 s and overlaid with 60 to 75 μl of mineral oil. Tube caps were closed tightly, and tubes were placed in a microwave polypropylene rack (BioVentures). The rack was placed in a microwave oven at 650 to 800 W for 6.25 min. The tubes were removed immediately after microwave heating.

For IC-PCR, sour cherry samples (leaf, bark, or petal tissue) were ground in citrate extraction buffer (50 mM sodium citrate, pH 8.3, containing 20 mM diethyldithiocarbamate (DIECA), 1 mM EDTA, and 2% [wt/vol] polyvinylpyrrolidone [PVP]); plum, peach, and apricot tissue samples were ground in phosphate-buffered saline (PBS)-Tween-2% PVP buffer (0.1 g of sample per 1 ml of buffer). Wells of Nunc-Maxisorp ELISA plates (Nunc Bio-Assay, Roskilde, Denmark) were precoated for 2 h at 37°C or overnight at 4°C with 200 μl of a 1:1,000 dilution of Bioreba-PPV (Bioreba, Basel, Switzerland) polyclonal antibody diluted in coating buffer (carbonate buffer, pH 9.6). Aliquots (200 μl) of plant extract were added to each well of the microplates. Microplates were covered with Parafilm and incubated overnight at 4°C in a plastic bag. After six washes with PBS-Tween-PVP buffer, aliquots (23 to 25 μl) of transfer buffer (10 mM Tris-HCl, pH 8.0, containing 1% Triton X-100) were added to each well, and the microplates were kept at 65°C for 5 min with periodic shaking.

RT-PCR amplification. Microwaved GR extract or IC solution (20 μl) was added to a 1.5-ml Eppendorf tube containing 10 μl of annealing reaction mixture: 6 μl of 5× RT buffer (0.25 M Tris-HCl, pH 8.3, 0.375 M KCl, 15 mM $MgCl_2$; Gibco-BRL Life Technologies, Gaithersburg, MD), 3 μl of 0.1 M dithioreitol (DTT), and 1 μl of complementary primer (1 μg/μl). The mixture was heated in boiling water for 5 min, placed on ice for 2 min, and left at room temperature for 45 to 60 min to allow primer annealing to the viral RNA template. cDNA synthesis was performed in the same tube after the addition of 4 μl of 5× RT buffer, 2.5 μl of 10 mM dNTPs, 5 μl of 0.3 M 2-mercaptoethanol, 2.0 μl of 0.1 M DTT, 1 μl of RNasin (40 units), 4.5 μl of sterile water, and 1 μl of Maloney murine leukemia virus reverse transcriptase (200 units). The 50-μl mixture was incubated for 1.5 to 2.0 h at 42°C.

Each PCR tube contained 5 μl of 10× PCR buffer (1× = 10 mM Tris-HCl, pH 8.3, 50 mM KCl, and 0.01% [wt/vol] gelatin), 3 μl of 25 mM $MgCl_2$, 1 μl of 10 mM dNTPs, 1 μl of each 6 μM (or 0.1 μg/μl) complementary and homologous primer, 0.5 μl of *Taq* DNA polymerase (Promega, Madison, WI), and sterile water to a final volume of 45 μl. Each reaction mixture was overlaid with 60 to 75 μl of mineral oil to prevent evaporation, was "hot started" (3) at 85°C for 5 min in a DNA thermocycler (Perkin-Elmer Cetus, Norwalk, CT), and 5 μl of the RT reaction mixture was added. Amplification was carried out for 30 cycles with the following parameters: denaturation at 94°C for 30 s, annealing at 62°C for 30 s, and extension at 72°C for 45 s, with a final extension at 72°C for 7 min.

Electrophoretic analysis of RT-PCR–amplified products. Aliquots (5 μl each) of RT-PCR–amplified products were analyzed by electrophoresis on 5% polyacrylamide gels at 120 to 150 V for 1.5 h in 1× TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM Na_2EDTA, pH 8.3) and visualized by staining with silver nitrate. DNA size markers (BioVentures) were used to determine the size of the amplified products. RFLP analysis of the amplified products was done with *Rsa*I restriction endonuclease (Gibco-BRL; 10 units/μl). Gel-purified DNA fragments (20) in a total volume of 20 μl were treated with 20 units of *Rsa*I for 2 h at 37°C. Samples were analyzed by polyacrylamide gel electrophoresis (PAGE).

Cloning of RT-PCR–amplified products. PCR products were eluted from polyacrylamide gels by the crush and soak method (20) and directly cloned into the pCR cloning vector (TA Cloning system, Invitrogen Corp., San Diego, CA). The pCR cloning vector is designed so the inserted PCR product is flanked on each side by an *Eco*RI restriction site. To detect the presence of the insert, *Eco*RI restriction analysis was done, followed by PAGE analysis.

Synthesis of the PPV cRNA probe. PPV cRNA was transcribed from the pCR cloning vector containing the PPV insert with T7 RNA polymerase and ^{32}P-labeled–UTP (specific activity 800 Ci/mmol). All steps were performed according to standard transcription protocol (Promega). A nonradioactive digoxigenin (DIG)-labeled T7 RNA polymerase-generated PPV cRNA probe also was synthesized by the Boehringer Genius nonradioactive system (Boehringer Mannheim, Indianapolis, IN).

Hybridization. Separated PCR products in polyacrylamide gels were denatured twice with 0.5 M NaOH and 1 M NaCl for 15 min and neutralized twice for 15 min with 0.5 M Tris-HCl, pH 7.4, and 1.5 M NaCl. Denatured DNAs were electrotransferred to Nytran membranes (Schleicher and Schuell, Keene, NH) in Tris-acetate-EDTA buffer at 0.6 A for 16 h at 4°C. Transferred DNA fragments were cross-linked to membranes by irradiation (1,200 μJ over 45 s) in a UV cross-linker (Strategene, La Jolla, CA). Membranes were prehybridized for 1 h at 55°C in prehybridization buffer (6× SSC [1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0], 50% formamide, 1% sodium dodecyl sulfate, 10× Denhardt's reagent, and 1 μg of calf thymus DNA per ml) and hybridized with the ^{32}P-labeled PPV cRNA probe (10^6 cpm/ml) or with the DIG-11–labeled probe (total of 500 ng) overnight at 55°C. Membranes were washed and treated with RNase A (1 μg/ml) for 15 min at 37°C to reduce background. Membranes hybridized with the ^{32}P-labeled probe were exposed to x-ray film (Amersham Hyperfilm; Amersham Corp., Arlington Heights, IL) for 24 h at –70°C. Membranes hybridized with the DIG-11–labeled probe were equilibrated for 1 min in Genius buffer 1 (100 mM maleic acid, 150 mM NaCl, pH 7.5) and soaked in Genius buffer 2 (10% blocking reagent dissolved in Genius buffer 1 and diluted 1:10) for 1 h with gentle shaking. Membranes were incubated in diluted anti-DIG alkaline phosphatase conjugate for 30 min. Hybridized products were visualized with the Genius nonradioactive detection kit with Lumi-Phos 530 substrate (Boehringer) and immediately overlaid with Hyperfilm-MP (Amersham) for 1 h.

Sequencing of cloned PCR products. Cloned PCR products (100 to 200 ng) were sequenced with a 5′-end–labeled primer in the fmol (Promega) sequencing system. The sequencing reaction was performed in a thermocycler with the following cycling parameters: denaturation at 94°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 45 s, for a total of 30 cycles. The Boehringer nonradioactive DIG *Taq* DNA-sequencing kit also was used according to manufacturer's directions.

Electron microscopy. Dried sour cherry anthers containing pollen and free pollen from PPV-infected and healthy sour cherry plants were processed for electron microscopy. Samples were fixed for 1 h in 5% glutaraldehyde in 0.1 M potassium phosphate, pH 7.2, washed twice for 15 min in phosphate buffer, and postfixed in 1% OsO_4. Dehydration was done for 15 min in 70, 85, 95, or 100% ethanol and 15 min in propylene oxide. Tissues were infiltrated with Epon 812 overnight in a mixture of 50% Epon and 50% propylene oxide, embedded in pure Epon, and cured at 60°C. Sections were cut with an ultramicrotome, stained with 5% uranyl-acetate for 20 min, stained with Reynold's lead citrate for 10 min, and examined with a Philips EM 300 electron microscope (Philips Electronic Instruments, Mahwah, NJ).

Chip bud transmission of PPV-SoC to cherry rootstocks. Experiments were carried out on two rootstocks of cherries: a mazzard clone of *P. avium* and a clone of *P. mahaleb*. PPV-infected sour cherry cultivar Orlovskaya Konservnaya was used for chip budding inoculation. Plants were maintained under containment at the U.S. Department of Agriculture quarantine facility in Beltsville, MD.

RESULTS

RT-PCR amplification of the 3′-NCR of the PPV-SoC genome from infected plant tissue. An amplified fragment of the expected size (220 bp) was obtained from PPV-infected sour cherry samples, whether the starting material was from leaf (Fig. 1A, lanes 3 through 5), flower, or seed tissue released from stone endocarp (data not shown). This product was similar in size to the product obtained from PPV-infected plum leaf tissue (Fig. 1A, lane 2), but it was absent in the uninfected sour cherry sample (Fig. 1A, lane 1). All the amplified 220-bp DNA fragments hybridized with a ^{32}P-labeled PPV cRNA probe for the 3′-NCR (Fig. 1B, lanes 2 through 5). No hybridization was obtained with samples from uninfected sour cherry tissue (Fig. 1B, lane 1).

Strain identity of PPV-SoC. To determine whether the PPV-SoC belongs to the D- or M-type PPV strains or whether it is a new undescribed strain of PPV, we utilized IC-RT-PCR assay (29). In this assay, a 243-bp cDNA fragment was amplified from the 3′-terminal region of the PPV CP gene and analyzed by RFLP. A cDNA fragment of the expected size (243 bp; Fig. 2) was amplified from PPV-infected sour cherry, apricot, plum (Fig. 2, lanes 2, 3, and 4, respectively) and peach (lanes 5 and 6) but not from healthy tissue (lane 7). A gel-purified 243-bp fragment of each PPV isolate was digested with the restriction endonuclease *Rsa*I and analyzed on 5% polyacrylamide gel. The restriction profile of the amplified fragments from PPV-infected apricot, plum, and peach showed the presence of an identical recognition site that was absent in the PPV-SoC cDNA (data not shown). The *Rsa*I recognition sequence, GTAC, is characteristic for isolates of the D group of PPV (28).

The nucleotide sequence of the cloned PCR-amplified cDNA fragment of the 3′-terminal region of the CP gene and the adjacent 3′-NCR of PPV-SoC agreed with RFLP analysis and revealed the absence of the *Rsa*I and the conserved *Alu*I sites at the expected positions in the PPV-SoC genome (Fig. 3A and B). In contrast, the conventional Moldovan plum PPV isolate nucleotide sequence corresponding to the 243-bp amplified fragment of the 3′-terminal region of the CP gene revealed the presence of both *Rsa*I and conserved *Alu*I restriction sites (data not shown). Comparison of the 450-nucleotide sequence of PPV-SoC with those of PPV-D (26), PPV-NAT (18), PPV-RAN (14), PPV-El Amar (27), and PPV-PS and PPV-06 (2) revealed nucleotide sequence identities of 91.5 to 94.2%. The calculated percentage of nucleotide sequence identity of each of the above PPV isolates to that of PPV-Soc was PPV-D = 91.5%; PPV-NAT = 94.2%; PPV-RAN = 93.7%; PPV-PS = 92.8%; PPV-06 = 92.4%; and PPV-El Amar = 91.5%. The nucleotide sequence identity of this region among members of the same viral group was as high as 98%.

Electron microscopy. Thin sections of anther tissue from PPV-SoC–infected and uninfected sour cherry flowers were examined by electron microscopy. Virus-encoded cytoplasmic inclusion bodies characteristic for the genus *Potyvirus* were found in infected cells of anther walls (Fig. 4) but not in pollen grains or uninfected tissue. The cylindrical inclusions were tubular scrolls and curved laminated aggregates.

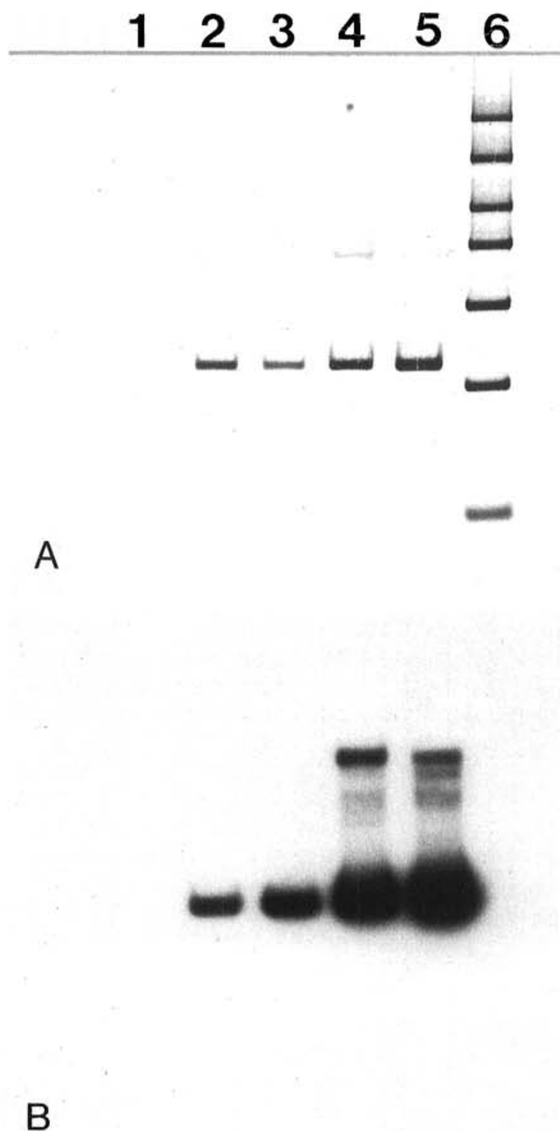

Fig. 1. A, Polyacrylamide gel electrophoretic analysis and **B,** Southern blot hybridization analysis with ^{32}P-labeled plum pox virus (PPV) cRNA probe of reverse-transcription polymerase chain reaction (RT-PCR)-amplified PPV cDNA from Gene Releaser-treated (BioVentures) extract of infected or uninfected leaf tissue. Lane 1, uninfected control; lane 2, PPV-infected plum; lanes 3 and 4, PPV-infected sour cherry 'Hybrid 8-34-3'; lane 5, PPV-infected sour cherry 'Orlovskaya Konservnaya'; lane 6, DNA size markers (1,000, 700, 500, 400, 300, 200, and 100 bp).

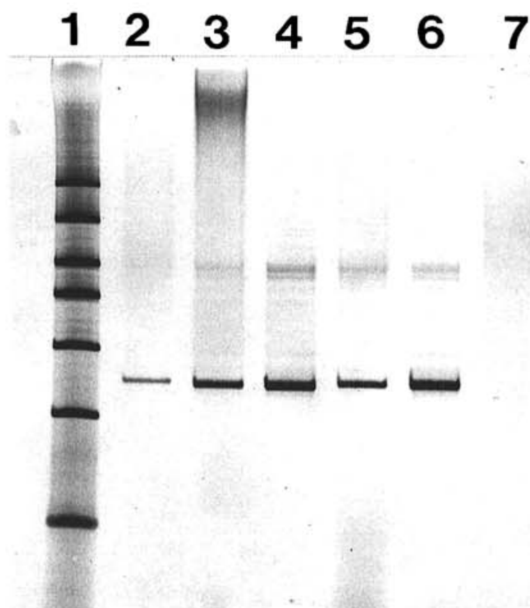

Fig. 2. Polyacrylamide gel electrophoretic analysis of amplified plum pox virus (PPV) cDNA from PPV-infected sour cherry, apricot, plum, and peach when primers for the 3′-terminal region of the PPV coat protein gene were used for amplification. Lane 1, DNA size markers (100 to 1,000 bp). PPV cDNA was amplified from: lanes 2 through 4, PPV-infected sour cherry, apricot, and plum, respectively; lanes 5 and 6, PPV-infected peach; and lane 7, uninfected peach.

Distribution of PPV-SoC in infected sour cherry trees. To determine whether PPV was localized or systemically distributed in infected sour cherry trees, different tissues were analyzed by RT-PCR with primers for the 3′-NCR of PPV. Most tissues from infected sour cherry cultivars Orlovskaya Konservnaya, Pandy, and Hybrid 8-34-3 assayed positive for PPV. Tested samples included leaf, bark, root, flower parts (pistils, stamens, filaments, anthers, pollen, sepals, and petals), seed, and fruit. PPV-SoC symptoms on fruit included depressions and rings. The rings gradually disappeared during fruit ripening.

Graft transmission of PPV-SoC to cherry rootstocks. Distinct leaf symptoms appeared on *P. avium* and *P. mahaleb* about 6 months after graft-inoculation by chip buds from infected sour cherry trees. Chlorotic spots appeared on mazzard leaves, and vein yellows with necrosis appeared on leaves of *P. mahaleb*. To verify the identity of the transmitted virus, we utilized RT-PCR for the amplification of PPV-specific cDNA. The 220- and 243-bp cDNA fragments were amplified from leaf tissue of infected *P. avium* and *P. mahaleb* with 3′-NCR primers and primers for the 3′-terminal region of the PPV CP gene, respectively. RFLP analysis of the amplified 243-bp cDNA from both plant species indicated the absence of a *Rsa*I restriction site. PPV infection in *P. avium* or *P. mahaleb* was not localized but was systemically distributed, as indicated by several RT-PCR assays of leaves collected from different locations on the rootstocks that showed the symptoms described above. One year after showing symptoms, leaves of PPV-Soc–infected *P. avium* and *P. mahaleb* plants were reassayed for PPV by IC-RT-PCR with primers for the PPV 3′-NCR and the CP gene. PCR assays indicated that PPV-SoC remained systemic in infected plants and did not localize or disappear from infected tissue in infected plants for up to 1.5 years after graft inoculation.

A

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CAGACTACAGCCTCGCCAGATATGCCTTTG 30
ATTTCTACGAGATGACCTCGACAACGCCTG 60
*             **
TGAGGGCTCGTGAGGCACATATTCAAATGA 90
AGGCAGCAGCATTGAGAAATGTTCAAAATC 120
GTTTATTTGGCTTGGATGGAAACGTCGAAA 150
CACAAGAAGAGGACACAGAGAGGCACACCG 180
CTGGTGATGTTAATCGCAACATGCACAACC 210
TCCTCGGTGTGAGGGGAGTGTAGTGGTCTC 240
GGTATCCATCATAAACTCTACTTGGGTGAG 270
AGTCTAGTCATCCAACTGTTTTAGATTCC 300
TGTTAGCATCCTTTTCTTTGCTTTAATAGC 330
AGTACATCCAGTGAGGTTTTACCTCCATAT 360
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B

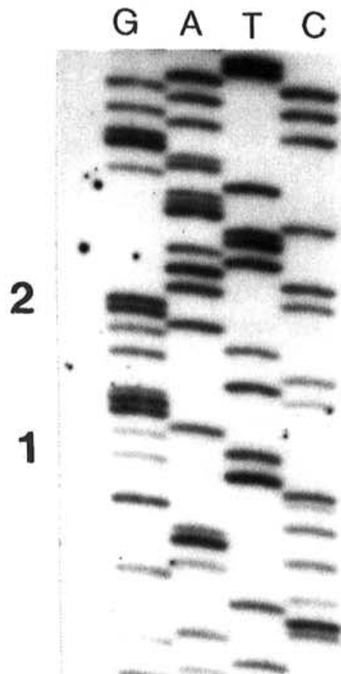

Fig. 3. A, Nucleotide sequence of the 3′-terminal region of the coat protein gene and 3′ noncoding region of plum pox virus sour cherry strain (PPV-SoC) and **B,** an autoradiograph of the sequencing gel. **A,** * and ** indicate the common positions of *Rsa*I (GTAC) and *Alu*I (AGCT) recognition sequences, respectively. **B,** 1 and 2 indicate the lack of *Rsa*I and *Alu*I recognition sequences, respectively.

DISCUSSION

This study demonstrated that the potyvirus infecting sour cherry trees in Moldova is indeed PPV and that it represents a unique strain of PPV and a prototype of a new group of PPV strains. The virus is distributed systemically in infected sour cherry trees and infects other cherry species. Our study also confirms and extends biological and serological studies that suggest natural infection of sour cherry with PPV is occurring in Moldova.

So far, PPV has been the only potyvirus known to infect stone fruits. Recently, however, a new virus, Asian Prunus latent virus, has been reported to infect *Prunus* spp. (9,10). This virus reacts positively with PPV CP antiserum, but its RNA does not anneal to primers specific for the 3′-NCR of PPV in RT-PCR assay (9). The current investigation established that sour cherry trees in Moldova were not infected with Asian Prunus latent virus. In addition, the IC-RT-PCR assay for the 3′-terminal region of the PPV CP gene has provided further evidence concerning the identity of the virus infecting sour cherry trees.

Using this assay, Wetzel et al. (28) showed that isolates of PPV can be classified into two groups, PPV-D and PPV-M, according to RFLP analysis of amplified products with the restriction enzyme *Rsa*I. Isolates of PPV-D contain a *Rsa*I recognition sequence, whereas those of PPV-M lack this sequence. The D- and M-type isolates represent the two distinctive and conventional serotypes of PPV (13,28). RFLP analysis of the PCR-amplified cDNA fragment from the 3′-terminal region of the CP gene of PPV-SoC as well as nucleotide sequence analysis of the cloned cDNA fragment revealed the absence of an *Rsa*I recognition sequence in RT-PCR products amplified from all three infected sour cherry cultivars studied. These results suggest that this isolate belongs to PPV-M; however, because PPV-SoC does not contain the conserved *Alu*I recognition sequence found in all PPV isolates sequenced thus far (2,14,18,26,27) and its cross-reactivity with CP antiserum for the PPV-D serotype is high (12), we suggest that this isolate is unusual among known isolates of PPV and should be considered a unique strain of PPV and a prototype of a new group, the PPV-C (cherry) group. This suggestion has been strongly supported by a recently obtained nucleotide sequence of the PPV-SoC CP gene (L. Nemchinov, E. Maiss, and A. Hadidi, *unpublished data*).

Detection of PPV in fruits, seeds, flowers, leaves, bark, and roots of infected trees indicates the virus is systemically distributed in infected trees. PPV was detected by RT-PCR in anthers and pollen from the same trees. Electron microscope examinations of anther and pollen, however, revealed that PPV-encoded "pinwheel" inclusion bodies were located in anther walls (tapetum cells), but no inclusion bodies were detected in pollen. This may suggest that a small portion of pollen grains was contaminated with PPV virions from infected anthers, which resulted in a positive RT-PCR assay for PPV. Pollen contamination most likely was due to breakdown of PPV-infected tapetal cells. PPV-infected anthers or contaminated pollen could be the source of virus dissemination during the international movement of stone fruit anther germ plasm collection for breeding programs or other purposes (16).

Under controlled conditions, we successfully transmitted PPV-SoC by chip bud grafting to *P. avium* and *P. mahaleb* rootstocks. Recently, natural infection of sweet cherry trees (*P. avium*) in southern Italy with PPV has been reported (4,5). The identity of this isolate (PPV-SwC) is currently under investigation. However, preliminary results from our laboratory suggest that both PPV-SoC and PPV-SwC isolates may be significantly different from conventional strains of PPV and may represent an undescribed group of PPV (A. Crescenzi, P. Piazzolla, and A. Hadidi, *unpublished data*). PPV-SoC was reported to be transmitted by chip budding to plum (12). Thus, PPV-SoC is not restricted to cherry species and has the potential to infect other stone fruits.

Currently, quarantine regulations in the United States and other countries do not require testing for PPV in foreign quarantined sour and sweet cherry germ plasm due to the fact that these plants are thought to be resistant or immune to infection with most, if not all, known D- and M-type strains of PPV. Our investigation, however, revealed the presence of a unique strain of PPV that systemically infects cherry plants. For this reason, we suggest that quarantine regulations should be changed to prevent the introduction of PPV through the international movement of infected cherry germ plasm.

PPV-SoC was most likely introduced into Moldova with infected sour cherry cultivars from Russia. PPV-infected cultivars Orlovskaya Konservnaya and Kistevaya were first brought into Moldova from the Research Institute of Fruit Growing and Breeding, Orlov, Russia, and from the Horticultural Research Institute, Michurinsk, Russia, respectively, during the early 1970s. Recently, PPV has been detected in sour cherry mother trees in Russia (12), which supports the hypothesis that PPV-SoC was introduced into Moldova in infected sour cherry germ plasm from Russia.

LITERATURE CITED

1. Candresse, T., Lanneau, M., Grasseau, N., Macquaire, G., German, S., Malinovsky, T., and Dunez, J. 1995. An immunocapture PCR assay adapted to the detection and the analysis of the molecular variability of apple chlorotic leaf spot virus. Acta Hortic. 386:136-147.
2. Cervera, M. T., Riechmann, J. L., Martin, M. T., and Garcia, J. A. 1993. 3′-terminal sequence of the plum pox virus PS and o6 isolates: Evidence for RNA recombination within the potyvirus group. J. Gen. Virol. 74: 329-334.
3. Chou, Q., Russel, M., Birch, D. E., Raymond, J., and Bloch, W. 1992. Prevention of pre-PCR and primers-dimerization improves low-copy number amplification. Nucleic Acids Res. 10:1717-1723.
4. Crescenzi, A., Nuzzaci, M., Levy, L., Piazzolla, P., and Hadidi, A. 1994. Infezioni di sharka su ciliegio dolce in Italia meridionale. Inform. Agrar. 34:73-75.
5. Crescenzi, A., Nuzzaci, M., Levy, L., Piazzolla, P., and Hadidi, A. 1995. Plum pox virus in sweet cherry. Acta Hortic. 386:219-225.
6. Dosba, F., Maison, P., Lansac, M., and Massonie, G. 1987. Experimental transmission of plum pox virus (PPV) to *Prunus mahaleb* and *Prunus avium*. J. Phytopathol. 120:199-204.
7. Dunez, J., and Sutic, D. 1988. Plum pox virus. Pages 44-46 in: European Handbook of Plant Disease. I. M. Smith, J. Dunez, R. A. Lelliot, D. H. Philips, and S. A. Archer, eds. Blackwell Scientific Publication, Oxford, MA.
8. Hadidi, A., Davis, I., Minafra, A., Boscia, D., and Postman, J. D. 1995. A virus affecting apples and pears is related to grapevine virus A as revealed by IC-RT-PCR and molecular hybridization. Acta Hortic. 386: 37-44.
9. Hadidi, A., and Levy, L. 1994. Accurate identification of plum pox virus and its differentiation from Asian Prunus latent potyvirus in *Prunus* germplasm. EPPO Bull. 24:633-643.

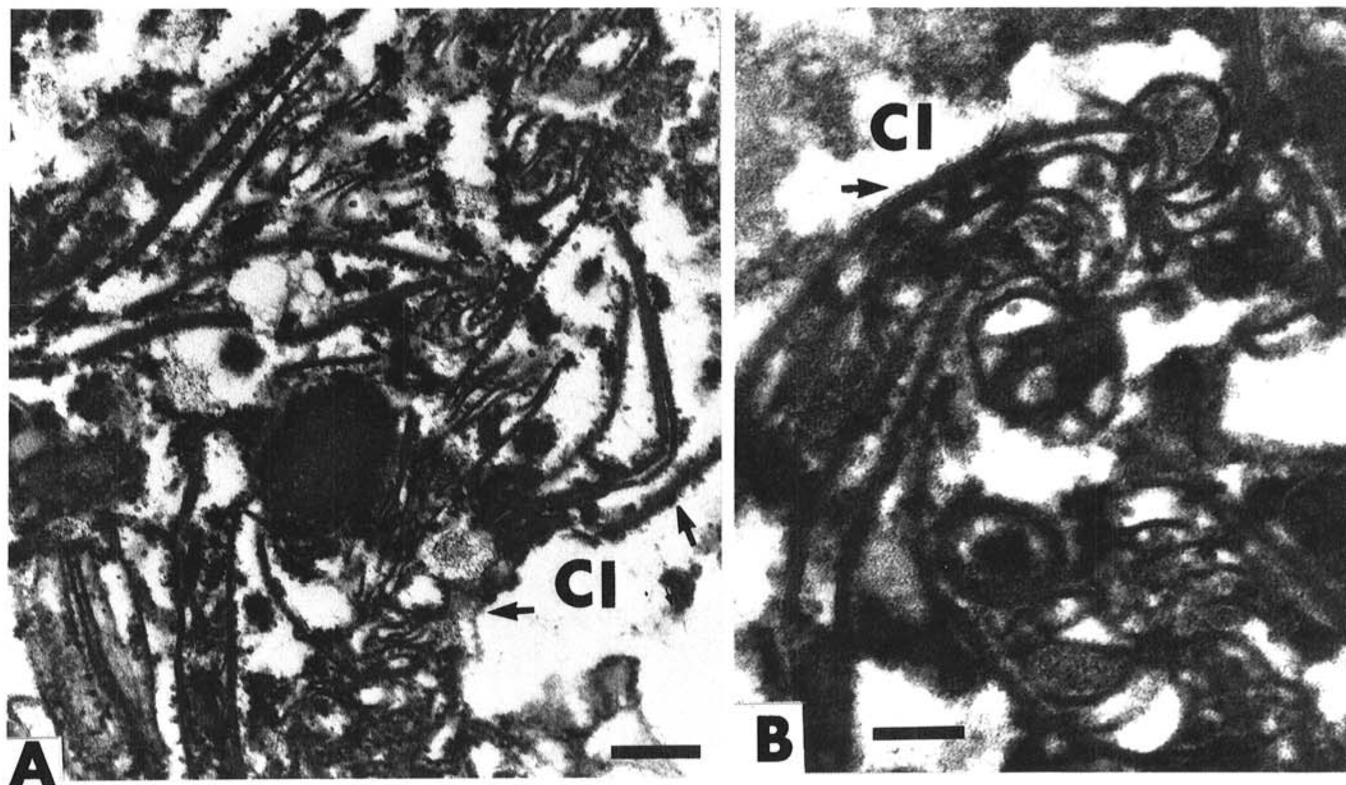

Fig. 4. Ultrathin sections of sour cherry anthers containing pollen obtained from a plum pox virus-infected tree. **A and B,** virus-encoded cylindrical cytoplasmic inclusions in infected anthers. Scale bars: **A,** 250 nm and **B,** 100 nm.

10. Hari, V., Abdel-Ghaffar, M. H., Levy, L., and Hadidi, A. 1995. Asian Prunus latent virus: An unusual potyvirus detected in germplasm from East Asia. Acta Hortic. 386:78-82.
11. Kalashyan, Yu. A., and Bilkey, N. D. 1989. Identifikatsiya virus sharky na vishne. Plant Virology. Page 106 in: (Abstr.) Proc. 10th Conf. Czechoslavak Plant Virol., Prague.
12. Kalashyan, Yu. A., Bilkey, N. D., Verderevskaya, T. D., and Rubina, E. V. 1994. Plum pox virus on sour cherry in Moldova. EPPO Bull. 24:645-649.
13. Kerlan, C., and Dunez, J. 1979. Differentiation biologique et serologique de souches du virus de la Sharka. Ann. Phytopathol. 11:241-250.
14. Lain, S., Reichmann, J. L., Mendez, E., and Garcia, J. A. 1988. Nucleotide sequences of the 3′ terminal region of plum pox potyvirus RNA. Virus Res. 10:325-334.
15. Levy, L., and Hadidi, A. 1994. A simple and rapid method for processing PPV-infected tissue for use with PPV-specific 3′ non-coding region RT-PCR assays. EPPO Bull. 24:595-604.
16. 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 Hortic. 386:331-339.
17. Levy, L., Lee, I.-M., and Hadidi, A. 1994. Simple and rapid preparation of infected plant tissue extracts for PCR amplification of virus, viroid and MLO nucleic acids. J. Virol. Methods 49:295-304.
18. Maiss, E., Timpe, U., Brisske, A., Jelkman, W., Casper, R., Himmler, G., Mattanovich, D., and Katinger, H. W. D. 1989. Complete nucleotide sequence of plum pox virus RNA. J. Gen. Virol. 70:513-524.
19. Minafra, A., and Hadidi, A. 1994. Sensitive detection of grapevine virus A, B, or leafroll associated III from viruliferous mealybugs and infected tissue by cDNA amplification. J. Viol. Methods 57:175-188.
20. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
21. Nemchinov, L., and Hadidi, A. 1995. Sour cherry isolate of plum pox virus is a unique member of the viral M strain and it is transmitted by chip budding to sour and sweet cherry. (Abstr.) Phytopathology 85:631.
22. Nemchinov, L., Hadidi, A., Candresse, T., Foster, J. A., and Verderevskaya, T. D. 1995. Sensitive detection of apple chlorotic leaf spot virus from infected apple or peach tissue using RT-PCR, IC-RT-PCR, or multiplex IC-RT-PCR. Acta Hortic. 386:51-62.
23. Nemchinov, L., Hadidi, A., and Verderevskaya, T. 1994. Plum pox virus in sour cherry: Confirmation of its identity by RT-PCR, cloning and nucleotide sequencing of the genomic 3′ non-coding region. Page 11 in: (Abstr.) Int. Conf. Fund. Appl. Problems Phytovirol., Yalta, Crimea, Ukraine.
24. Nemchinov, L., Hadidi, A., and Verderevskaya, T. 1995. Detection and partial characterization of a plum pox virus isolate from infected sour cherry. Acta Hortic. 386:226-237.
25. Nemeth, M. 1986. Virus, mycoplasma, and rickettsia diseases of fruit trees. Akademia Kiado, Budapest.
26. Teycheney, P. Y., Tavert, G., Delbos, R. P., Ravelonandro, M., and Dunez, J. 1989. The complete nucleotide sequence of plum pox virus RNA (strain D). Nucleic Acids Res. 17:10115-10116.
27. Wetzel, T., Candresse, T., Ravelonandro, M., Delbos, R. P., Mazyad, H., Aboul-Ata, A. E., and Dunez, J. 1991. Nucleotide sequence of the 3′-terminal region of the RNA of the El Amar strain of plum pox potyvirus. J. Gen. Virol. 72:1741-1746.
28. Wetzel, T., Candresse, T., Ravelonandro, M., and Dunez, J. 1991. A polymerase chain reaction assay adapted to plum pox potyvirus detection. J. Virol. Methods 33:355-365.
29. Wetzel, T., Candresse, T., 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.