MOLECULAR EVIDENCE FOR THE OCCURRENCE OF PLUM POX VIRUS - CHERRY SUBGROUP IN HUNGARY

L. Nemchinov and A. Hadidi
Fruit Laboratory
Agricultural Research Service
United States Department of Agriculture
Beltsville, MD. 20705,
USA

M. Kölber and M. Németh
Plant Health and Soil Conservation Station
of the Ministry of Agriculture
Budapest Budaöri ut 145,
Hungary

Abstract

Leaf samples from peach GF 305 seedlings graft-inoculated with bud chips from sweet cherry trees cv. 'Van', which tested positive for plum pox virus (PPV) by ELISA, were analyzed by molecular methods for the presence or absence of PPV-Cherry (PPV-C). These analyses indicated that the sweet cherry cultivar is infected with a member of the established PPV-C subgroup as shown by RT-PCR amplification of PPV cDNA of the expected size using DNA primers specific for PPV-C and the demonstration that nucleotide sequence analysis of the cloned RT-PCR product contains the distinct 5'-terminal sequence of the coat protein coding region, characteristic for PPV-C members. Our results also suggest that PPV-C may be more widespread in eastern Europe than was originally thought.

1. Introduction

During the last few years, sweet and sour cherry trees were found to be natural hosts for a unique strain of plum pox virus (Crescenzi et al., 1994, 1995; Kalashyan et al., 1994; Nemchinov et al., 1995). A new subgroup of PPV strains namely PPV-Cherry (PPV-C), which significantly differs from the conventional D and M strains and from the El-Amar strain, has been established (Crescenzi et al., 1997; Nemchinov and Hadidi, 1996; Nemchinov et al., 1996, 1998). The status of PPV-C distribution and its economic significance currently have not been defined. However, reports from Italy, Bulgaria, and an unconfirmed communication from Russia support the possible widespread occurrence of PPV-C in Europe (Crescenzi et al., 1997; Kalashyan et al., 1994; Topchijska, 1996).

In Hungary, several hundred sweet and sour cherry trees tested positive for PPV by ELISA and chip buds from these trees were grafted onto GF 305 peach seedlings (Kölber et al., 1998).

It is known that serology using polyclonal antibodies might be an unsatisfactory approach for the identification of potyviruses (Shukla and Ward, 1989). PPV antiserum has been reported to cross-react with other potyviruses and viruses of unknown origin ( Hadidi and Levy, 1994; James et al., 1996). To determine whether the virus infecting cherry trees in Hungary is indeed PPV, and more specifically, PPV-C, we have utilized PCR technology, molecular cloning and sequencing to analyze plant samples from sweet and sour cherry trees as well as graft-inoculated peach seedlings. Here we report molecular evidence for the occurrence of PPV-C in Hungary.

2. Materials and methods

2.1. Source of plant tissue

Leaf samples from graft-inoculated peach GF 305 seedlings were obtained from the Plant Health and Soil Conservation Station, Budapest, Hungary and then processed in the U.S. for molecular analysis.
2.2. Preparation of samples for IC-RT-PCR, cloning and sequencing

Immunocapture of viral particles was used in most cases to release viral RNA from infected tissue (Wetzel et al., 1992). The procedure was performed as described elsewhere (Nemchinov and Hadidi, 1996) with PPV polyclonal antiserum (Sanofi, France) diluted 1:1000. Two sets of PPV-specific primers were used for RT-PCR in this investigation: (i) universal primers, derived from the 3' non-coding region (3' NCR) of PPV (Levy and Hadidi, 1994; Hadidi and Levy, 1994); and (ii) PPV-Cherry-specific primers HSoC2/CSoC2 (Nemchinov and Hadidi, 1998). The PCR parameters were as previously described except that the number of cycles was increased to 35 for amplification with PPV-C primers. PCR products were analyzed on polyacrylamide gels and stained with silver nitrate or ethidium bromide. Amplified viral cDNA was cloned into pCR II or pCR 2.1 vectors (Invitrogen Corp., San Diego, CA) and sequenced at the University of Maryland, College Park, MD, by ABI-PRISM 373A Genetic Analyzer (Applied Biosystems, Foster City, CA). Nucleotide sequences were aligned by the CLUSTAL method using DNASTAR LaserGene software (DNASTAR, Inc., Madison, WI).

3. Results

3.1. Detection of PPV-C from Hungary by IC-RT-PCR

When universal primers for the 3' NCR of PPV or primers specific for PPV-C were used in IC-RT-PCR assays, the symptomatic leaves of a GF 305 peach seedling, which was grafted on sweet cherry cv. 'Van', were PPV-positive. With either pair of primers, the amplified product was of the expected size but faint (not shown). A second round PCR, however, significantly increased the amount of PPV-specific cDNA amplified with either set of primers (Fig. 1).

3.2. Nucleotide sequence analysis

Nucleotide sequence analysis of cloned PCR products demonstrated that the virus infecting sweet cherry cv. 'Van' in Hungary is PPV. The 3' NCR shares about 95% homology with those of other PPV isolates, including the sour cherry isolate from Moldova (Fig. 2). In addition, analysis also showed that the virus is a member of the established PPV-C subgroup as it has the distinct 5'-terminal sequence of the coat protein coding region, characteristic for the PPV-C subgroup (Fig. 3).

4. Discussion

Our results demonstrated that the virus detected in cherry trees in Hungary is indeed PPV and is a member of the PPV-C subgroup. The virus has the distinct 5' - terminal sequence of the coat protein coding region, characteristic for the PPV-C subgroup. Nucleotide sequence data suggest that changes in the N-terminus of the viral coat protein are most likely responsible for the infection of cherry with PPV. Along with present findings in Moldova, Italy, and Hungary, PPV in sweet and sour cherry has been reported from Bulgaria, where a surprisingly high percentage of sour cherry trees were found to be infected with PPV using ELISA. Verification of these results with current molecular methods, however, has not yet been done (Topchija, 1996). PPV in cherry also may be present in central Russia; Kalashyan et al., 1994) reported that a polyclonal antiserum against PPV-SoC reacted positively in ELISA with cherry samples from this region. Thus, it seems that PPV-C may be more widespread in Europe than was originally thought.

References

Hadd Te of plum pox p 633-643.
Kolb M., Nem f use with P 633-643.
Levy L. and Had for use with P 633-643.
Shulka D.D. and the taxonomy Topchija M., 1 Meeting on Pl 39: 2
Wetzel T., Can immuno...
To release viral RNA from infected tissue as described elsewhere (Sanofi, France) diluted with Tris-EDTA buffer (pH 7.4) in this investigation: (i) universal oligonucleotide primers HScC2/ScC2 (NCR) of PPV (Levy and Hadidi, 1994) were previously described except that the primers are used with PPV-C primers. PCR products were electrophoresed in 1.5% agarose and stained with silver nitrate or ethidium bromide. The NCR primes (NCR 2.1 vectors (Invitrogen Corp., Carlsbad, CA) were amplified, Center for Agricultural Biotechnology and Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) were used for reaction analysis.

Specific for PPV-C were used to induce a transcript, which was grafted on a 3' - terminal sequence of the cDNA. With either pair of primers, a PCR product was obtained (not shown). A second primer pair was designed on the basis of cDNA amplified from the cherry isolate from Moldova (Nemchinov et al., 1996). Both primers were designed on the basis of the established PPV-C coat protein coding region, and used to amplify was NCR for the grapevine isolate from Moldova and the PPV-C isolate from Bulgaria.

The analysis of the NCR of PPV-C isolated from Moldova demonstrated that the virus isolated from Moldova (Nemchinov et al., 1996) and the NCR of the cherry isolate from Moldova (Nemchinov et al., 1996) are of the established PPV-C coat protein coding region, and used to amplify the NCR for the grapevine isolate from Moldova and the PPV-C isolate from Bulgaria.

The analysis of the NCR of PPV-C isolated from Moldova demonstrated that the virus isolated from Moldova (Nemchinov et al., 1996) and the NCR of the cherry isolate from Moldova (Nemchinov et al., 1996) are of the established PPV-C coat protein coding region, and used to amplify the NCR for the grapevine isolate from Moldova and the PPV-C isolate from Bulgaria.

References


Topolski M., 1996. Plum pox virus in some Prunus spp. in Bulgaria. In: Middle European Meeting on Plum Pox, Budapest, 2-4 October Pp.27.

Figure 1 - Polyacrylamide gel electrophoresis of immunocapture-RT-PCR-amplified cDNA from leaf extracts of GF-305 peach seedling, graft-inoculated with chip buds from PPV-ELISA-positive sweet cherry cv. 'Van'. Lane 1: H₂O (negative control); lanes 2 and 3: primers for the 3' non-coding region of PPV were used for IC-RT-PCR with uninfected and infected tissue, respectively; lanes 4 and 5: PPV-C- specific primers (HsoC2/CSoC2) were used for IC-RT-PCR with uninfected and infected tissue, respectively; M: BioLow™ DNA size markers (BioVentures, Murfreesboro, TN, USA)- 1000, 700, 525, 500, 400, 300, 200, and 100 bp.
Figure 2 - Multiple alignment of the 3' non-coding region sequence of the Hungarian PPV-C isolate with the corresponding sequence of seven different PPV isolates. Residues that differ from the HUN PPV-C are boxed.
Figure 2 - continued

1 TCCAACATCCCACAAATCTGCAGAGCGGGC PPV-C HUNGARY
1 TCCAACATCCCACAAATCTGCAGAGCGGGC PPV-SoC MOL
31 ACCAATGTGTGTTGATCCCATATCTCCACCTCAGC PPV-C HUNGARY
31 ACCAATGTGTGTTGATCCCATATCTCCACCTCAGC PPV-SoC MOL
61 AACACACCCAGCCAATGCGAGAGCCGATTCG PPV-C HUNGARY
61 AACACACCCAGCCAATGCGAGAGCCGATTCG PPV-SoC MOL
91 ACCAGTAGTGACAAGTCCTCATTCCTCAGTATGG PPV-C HUNGARY
91 ACCAGTAGTGACAAGTCCTCATTCCTCAGTATGG PPV-SoC MOL
121 GGTAAATTGGGAAACCAAGAAGGTGACACCTCC PPV-C HUNGARY
121 GGTAAATTGGGAAACCAAGAAGGTGACACCTCC PPV-SoC MOL
151 CCTCCCACAACTGCAAGTCCACAGGAAAGGPPV-C HUNGARY
151 CCTCCCACAACTGCAAGTCCACAGGAAAGGPPV-SoC MOL
181 GGATCGAGATGTA PPV-C HUNGARY
181 GGATCGAGATGTA PPV-SoC MOL

Figure 3 - Nucleotide sequence alignment of the 5'-terminal coat protein region of the Hungarian PPV-C with the corresponding sequence of the Moldovan strain of PPV from sour cherry (PPVSoC). The hyphens were introduced by the program to maximize the alignment.