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

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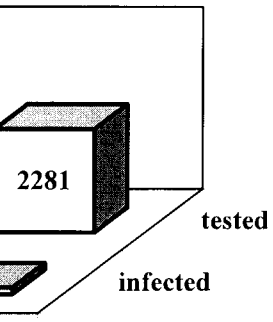

ISA in Hungary (1992-1996)

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 wide-spread 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.

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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, Center for Agricultural Biotechnology, 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 graft-inoculated with chip buds from 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 (Topchijska, 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 wide spread in Europe than was originally thought.

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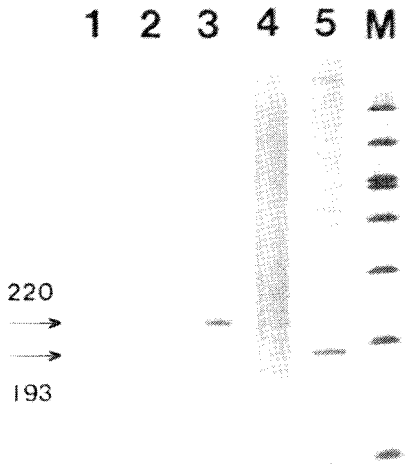

Figure 1 - Polyacrylamide gel electrophoresis of immunocapture-RT-PCR-amplified cDNA from leaf extracts of GF-305 peach seedling, graft-inoculated with chip buds from PPV-ELISA-positive sweet cherry cv. 'Van'. Lane 1: H_2O (negative control); lanes 2 and 3: primers for the 3' non-coding region of PPV 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.

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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.

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161 C T T G T A T C T G A T G T A G C G A G T G T T T C A C T C C A T T C G G G T T  HUN PPV-C 3'NCR
161 C T T G T A T C T G A T G T A G C G A G T G T T T C A C T C C A T T C G G G T T  PPV-SoC 3'NCR
159 C T T G T A T C T G A T G T A G C G A G T G [C] T [C] C A C T [T] C A T T C G G G T T  PPV-D 3'NCR
161 C T T G T A T C T G A T G T A G C G A G T G [C] T T C A C T C C A T T C G G G T T  PPV-RANW 3'NCR
161 C T T G T A T C T G A T G T A G C G A G T G T T T C A C T C C A T T C G G G T T  PPV-NAT 3'NCR
161 C T T G T A T C T G A T G T A G C G A G T G [C] T T C A C T C C A T T C G G G T T  PPV-AT 3'NCR
160 C T T G T A T C T G A T G T A G C G A G T G [C] T T C A C T C C A T T C G G G T T  PPV-EL 3'NCR
161 C T T G T A T C T G A T G T A G C G A G T G T T T C A C T C C A T T C G G G T T  PPV-Ps 3'NCR

201 A T A G T T C T T G T G C A A G A G A C [                                ]  HUN PPV-C 3'NCR
201 A T A G T T C T T G T G C A A G A G A C [                                ]  PPV-SoC 3'NCR
199 A T A G T T C T T G T G C A A G A G A C [                                ]  PPV-D 3'NCR
201 A T A G T T C T T G T G C A A G A G A C [                                ]  PPV-RANW 3'NCR
201 A T A G T T C T T G T G C A A G A G A C [                                ]  PPV-NAT 3'NCR
201 A T A G T T C T T G T G C A A G A G A C [                                ]  PPV-AT 3'NCR
200 A T A G T T C T T G T G C A A G A G A C [                                ]  PPV-EL 3'NCR
201 A T A G T T C T T G T G C A A G A G A C [                                ]  PPV-Ps 3'NCR
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Figure 2 - continued

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1   T C C A C C A T T C C C A A A T C T G C A G A G C A C G G C  PPV-C HUNGARY
1   T C C A C C A T T C C C A A A T C T G C A G A G C [G] C G G C  PPV-SoC MOL

31  A C C A A T G T T T G A T C C C A T A T T C A C T C C A G C  PPV-C HUNGARY
31  A C C A A T G T T T G A T C C C A T A T T C A C T C C A G C  PPV-SoC MOL

61  A A C A A C C C A G C C A A A T G C G A G A C C G A T T G C  PPV-C HUNGARY
61  A A C A A C C C A G C C A A A T G [T] G A G A C C G A T T G C  PPV-SoC MOL

91  A C C A G T A G T G A C A A G T C C A T T C T C G T A T G G  PPV-C HUNGARY
91  A C C A G T A G T G A C A A G T C C A T T C T C G T A T G G  PPV-SoC MOL

121 G G T A A T T G G G A A C C A G A A C G T G A C A C C T T C  PPV-C HUNGARY
121 G G T A A T T G G G A A C C A G A A C G T G A C A C C T T C  PPV-SoC MOL

151 C T C C C C A A A T G C A C T A G T C A A C A C G A G G A A  PPV-C HUNGARY
151 C T C C [T] C A A A T G C A C T A G T C A A C A C G A G G A A  PPV-SoC MOL

181 G G A T C G A G A T G T A [                    ]  PPV-C HUNGARY
181 G G A T C G A G A T G T A [                    ]  PPV-SoC MOL
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Figure 3 - Nucleotide sequence alignment of the 5'-terminal coat protein region of the Hungarian PPV-C with the corresponding sequence of the Moldovian strain of PPV from sour cherry (PPVSoC). The hyphens were introduced by the program to maximize the alignment.