Detection of *Plum Pox Virus* in Ornamental *Prunus cerasifera*

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*Plum pox virus* (PPV) is the causal agent of sharka disease. It is a serious threat for temperate fruit, mainly apricots, plums and peaches. In order to study the ability of PPV to infect wild and ornamental *Prunus* species, several wild, native ornamental stone fruits and weeds were analyzed as possible reservoirs of PPV. Five species of ornamental stone fruits and 24 species of weeds were evaluated between 2000 and 2004. The virus was not found in the weeds but was detected in one species of ornamental stone fruit, purple cherry-plums (*Prunus cerasifera Pissardii*). The PPV strain M was identified by DASI-ELISA and confirmed by IC-RT-PCR. Additionally, mealy plum aphid (*Hyalopterus pruni*) was determined as a vector of PPV in *P. cerasifera*. This is the first report on the reservoir potential of ornamental stone fruit trees and weeds for PPV in Turkey.

**KEY WORDS:** PPV; ELISA; IC-RT-PCR; epidemiology; ornamental *Prunus*; *Prunus cerasifera*; *Hyalopterus pruni*.

**INTRODUCTION**

Sharka disease caused by *Plum pox virus* (PPV) is the most detrimental viral disease of stone fruits. Numerous *Prunus* as well as herbaceous species are known to be PPV hosts (18). Parks, private gardens and road sides are often planted with some ornamental *Prunus* species. These areas may also contain some of the herbaceous host plants, which may be infected with PPV (13,18). These areas may pose an environmental risk to commercial orchards or nurseries located in the vicinity of these uncontrolled viral reservoirs.

Sharka disease was first reported in Bulgaria in 1917 in the plum variety 'Kjustendil'. The natural hosts of PPV were identified in the 1960s when much knowledge of this dangerous virus was obtained through experimentation in the former Yugoslavia as well as Hungary and Germany (17). PPV was discovered later in some ornamental *Prunus* species such as *P. cerasifera Pissardii*, *P. mahaleb*, *P. laurocerasus*, *P. spinosa*, *P. tomentosa*, *P. glandulosa* and *P. salicina* (18). Researchers discovered a large number of herbaceous plants such as *Senecio vulgaris*, *Ranunculus arvensis*, *Hyoscyamus niger*, *Physalis floridana*, *Solanum dulcamara*, *Pisum sativum*, *Melilotus officinalis*, *Trifolium repens* from different families, that were reported as hosts of sharka disease (18). The list of PPV host species cannot be considered definitive since new host species are continuously being detected. For example, Polak found PPV-infected *Euonymus europea* and *Ligustrum vulgare* (21); and Milusheva and Rankova confirmed the detection of PPV in *Capsella bursa-pastoris*, *Lactuca serriola*, *Lythospermum arvensis*, *Rumex crispus* and *Veronica hederifolia* samples, collected in plum and apricot orchards (15). These findings were
based on the use of ELISA with polyclonal antisera. Although using ELISA is the established method in the routine diagnosis of plant viruses, cross-reactions can occur between potyviruses (22,23). Therefore, to confirm positive serological results, molecular methods should be used for the detection of PPV in weeds (13).

Little is known about the role of weed species in the spread of sharka, although some authors (9,16) consider herbaceous plants to be a continuous source of infection for fruit species. Thus, we investigated the relationship between the economical use of ornamental Prunus, the possible detection of PPV in weeds, and the eventual transmission of PPV to commercial stone fruits by aphid vectors.

PPV has a limited distribution in Turkey, but it is commonly found to be present in apricot, plum and peach trees in Ankara. PPV-M is the predominant strain (7). Although PPV is widespread, no research has been done regarding ornamental stone fruit trees and weeds as potential hosts. This study was carried out to assess the natural spread of PPV in ornamental Prunus and weeds in Ankara.

MATERIALS AND METHODS

Ornamental stone fruits and weeds were evaluated for the presence of PPV infection in different areas of Ankara where PPV is widespread (7). The surveys for PPV incidence were conducted in April and June from 2000 to 2004. A total of 322 samples of ornamental Prunus and 250 samples of weed species were investigated.

All the samples were tested by the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) technique (5) using polyclonal antibody to determine PPV infection. To identify the PPV strain, the positive samples found by DAS-ELISA were subjected to double antibody sandwich indirect enzyme-linked immunosorbent assay (DASI-ELISA) (4) using PPV-M, PPV-D and PPV-EA type specific monoclonal antibodies, followed by the immunocapture reverse transcription-polymerase chain reaction (IC-RT-PCR) test (25).

IC-RT-PCR was used with some modifications. PCR tubes were coated with polyclonal immunoglobulins (100 µl) diluted in carbonate buffer [1/1000 (v/v)] and incubated overnight at 4°C. The tubes were washed once with PBS-Tween. Plant extracts were prepared by grinding the leaves in PBS containing 2% PBS-40 (1/10 w/v) and then centrifuged at 1000 x g for 3 min. The extracts (100 µl) were added to the tubes and incubated in ice for 3 h. The tubes were washed three times with PBS-Tween and then once with 20 mM Tris-HCl, pH 8.0.

For PCR, tubes contained 5 µl of 10 x PCR buffer, 2 µl of 10 mM dNTPs, 3 µl of 25 mM MgCl2, 1 µl of 20 mM each of the two primers, 0.5 µl of 40 u RNAse inhibitor, 0.2 µl of 200 u/µl reverse transcriptase, 0.5 µl of 5 u/µl Taq DNA polymerase and sterile water at a final volume of 50 µl. PPV primers were 5'-bio-ACGACACCGC-TACGGGCA-3' (for D-isolates) and 5'-bio-ACACACGCCTGTGCGTGCA-3' (for M-isolates) designed by Poggi-Pollini et al. (20). Reaction mixtures were incubated at 37°C for 1 h followed by 40 cycles of 94°C for 30 s, 54°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 10 min. PCR products were analyzed by 1.5% agarose gel electrophoresis in TBE buffer.

To study the possible aphid involvement in PPV spread, aphid transmission tests were conducted (6,8). GF 305 peach seedlings were preferred for the transmission tests because they are very susceptible to a large number of woody plant viruses including PPV (3).