

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 DAS-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 roadsides 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 europeae* 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

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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 μ l) 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 μ l) 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 μ l of 10 x PCR buffer, 2 μ l of 10 mM dNTPs, 3 μ l of 25 mM MgCl₂, 1 μ l of 20 pM/ μ l each of the two primers, 0.5 μ l of 40 u RNase inhibitor, 0.2 μ l of 200 u/ μ l reverse transcriptase, 0.5 μ l of 5 u/ μ l Taq DNA polymerase and sterile water at a final volume of 50 μ l. *PPV* primers were 5'-bio-ACGACACCCG-TACGGGCA-3' (for D-isolates) and 5'-bio-ACAAC-GCCTGTGCGTGCA-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). In

the test, 15 peach seedlings and 750 wingless *Hyalopterus pruni* aphids (600 viruliferous and 150 nonviruliferous) were used. A large number of aphids are required to reach good effectiveness of aphid transmission tests; therefore, 30–50 aphids per plant were required to assess the inoculation (14). For each aphid-infested *P. cerasifera*, aphid transmission tests were carried out on four GF 305 seedlings. The control seedlings were infested with non-infected aphids. The aphids which were fed on symptomatic leaves of *P. cerasifera* trees were disturbed, so that their stylets would be withdrawn. This was done by carefully tapping the abdomen with the aid of a small brush. Individual wingless aphids were carefully picked up, using the tip of the moistened brush, then immediately transferred (50 aphids/plant) to five leaves of healthy GF 305 seedlings (10 aphids/leaf). After 24 h, the peach seedlings were sprayed with a pesticide before putting them back into an insect-proof greenhouse. Inoculated plants were grown under controlled conditions (18–26°C) in an insect-proof greenhouse and checked periodically for the appearance of symptoms.

RESULTS AND DISCUSSION

Between 2000 and 2004, 322 ornamental *Prunus* trees were sampled in 15 parks, 52 private gardens and a few roadsides in Ankara. *Prunus cerasifera* (purple cherry plum) is the most common ornamental tree found in this area. Other ornamental species include: *P. laurocerasus* (cherry laurel), *P. mahaleb* (mahaleb cherry), *P. serrulata* (Japanese cherry) and *P. spinosa* (blackthorn). At the end of the 5-year study period, *PPV* had been found in only three of 322 samples (Table 1).

TABLE 1. The common ornamental stone fruit trees and the number of *PPV*-infected plants in this study

Sub-Genus	Species	Total no. of trees	No. of infected trees	<i>PPV</i> strain		
				M	D	EA
Prunophora	<i>P. cerasifera</i>	214	3	3	-	-
	<i>P. serrulata</i>	15	-	-	-	-
	<i>P. spinosa</i>	21	-	-	-	-
Laurocerasus	<i>P. laurocerasus</i>	37	-	-	-	-
Cerasus	<i>P. mahaleb</i>	35	-	-	-	-
Total		322	3	3	-	-

The symptoms observed were found most commonly on leaves located on the bottom branches of *P. cerasifera*. The symptoms of infection included red colored lines followed by slight distortion of the leaves most often observed in the spring. However, the symptoms were not obvious when leaves were fully expanded because of the masking effect of the intense color of the mature leaves. Therefore, it was difficult to visually detect infected trees. Flowers did not exhibit any symptoms, nor was any *PPV* detected in flowers.

To study the possible involvement of aphids in *PPV* spread, *P. cerasifera* was used in this study. Aphids could not be observed in any ornamental *Prunus* except for this one. On this plant the only rootstock sucker to be found was the mealy plum aphid, *H. pruni*. These rootstock suckers showed typical *PPV* symptoms. Elibuyuk (6) found that sharka disease was introduced into the healthy gardens by *H. pruni*. Aboul-Ela *et al.* (1) indicated that *H. pruni* was more efficient in transmitting *PPV* to apricots than *Myzus persicae*. Others found that *H. pruni* was moderately efficient to relatively ineffective as a vector (6). Conversely,

TABLE 2. Weeds which were collected in PPV-infected gardens

Family	Species	No. of samples	Symptoms
Amaranthaceae	<i>Amaranthus albus</i>	8	-
Apiaceae (Umbelliferae)	<i>Bifora radians</i>	5	-
	<i>Daucus carota</i>	11	-
Asteraceae (Compositae)	<i>Acroptilon repens</i>	14	-
	<i>Cirsium arvense</i>	11	-
	<i>Cichorium intybus</i>	10	-
	<i>Lactuca serriola</i>	12	-
	<i>Taraxacum officinale</i>	14	-
	<i>Xanthium strumarium</i>	11	-
Brassicaceae (Cruciferae)	<i>Capsella bursa-pastoris</i>	17	-
	<i>Sinapis arvensis</i>	19	LD, VB
	<i>Sisymbrium officinale</i>	12	LD, VB
	<i>Thlaspi arvense</i>	8	-
Chenopodiaceae	<i>Chenopodium album</i>	11	-
Convolvulaceae	<i>Convolvulus arvensis</i>	13	-
Lamiaceae	<i>Lamium amplexicaule</i>	11	-
Malvaceae	<i>Malva neglecta</i>	7	-
Plantaginaceae	<i>Plantago lanceolata</i>	8	-
Poaceae (Gramineae)	<i>Agropyron repens</i>	11	-
	<i>Digitaria sanguinalis</i>	7	-
	<i>Phragmites communis</i>	11	-
Polygonaceae	<i>Polygonum aviculare</i>	8	-
Solanaceae	<i>Datura stramonium</i>	6	-
	<i>Solanum nigrum</i>	5	-
Total		250	

LD, Leaf deformation; VB, Vein banding.

Labonne *et al.* (10) reported that *P. cerasifera* was the most difficult among the candidate studies in terms of the effectiveness of aphid transmission, but not for grafting. It is known that aphid vector species and their efficiency in transmitting viruses will vary according to climatic conditions, hosts and cultivars (2,11,12).

PPV symptoms on GF-305 seedlings appeared 64 days after the aphid inoculation. All 12 of the aphid-inoculated peach seedlings showed distorted or twisted leaves with vein clearing. These plants reacted positively in DAS-ELISA, which showed that the aphids which fed on infected trees were viruliferous and PPV was transmitted efficiently by the aphids to the seedlings. This finding proves that the three infected *P. cerasifera* trees have a role to play as a PPV source.

Three samples reacted with only the PPV-M type MABs. All positive PPV-M ELISA samples were retested by IC-RT-PCR using PPV-M and PPV-D primers. The samples produced the specific band for PPV-M in IC-RT-PCR. An amplified fragment of the expected size (243 bp) was obtained from PPV-infected samples. No DNA band was observed in buffer and healthy controls. PPV-M is considered to be the epidemic form of the virus and it spreads more readily by aphid vectors than by PPV-D isolates (6).

A total of 250 samples of weed species were sampled in 20 predominantly PPV-M-infected home gardens in different locations throughout Ankara. The weed species that were collected in these gardens are shown in Table 2. No symptoms were observed in the weeds with the exception of *Sinapis arvensis* and *Sisymbrium officinale*, in which only the *Cucumber mosaic virus* was detected (Table 2).

In this study, PPV could not be detected in any of the 250 samples collected. Similarly,

in the years 2000 and 2001, 16,855 samples of weed and ornamental species, collected in infected orchards and their close surroundings, were tested and none of them showed the presence of *PPV* (19). This differs from the findings of others, including: Milusheva and Rankova (15), who confirmed *PPV* infections in *C. bursa-pastoris* and *L. serriola* samples collected in plum and apricot orchards; and Marn *et al.* (13), who detected *PPV* in *T. officinale*, *Cichorium* sp., *C. arvense*, *S. arvensis* and *S. nigrum*. Furthermore, van Oosten (24) showed that *L. amplexicaule* and *S. nigrum* could be artificially infected with *PPV*. The absence of infection in the weeds may result from the absence of vector aphids and root connections. The mealy plum aphid (*H. pruni*) is the only aphid vector of *PPV* in Ankara which feeds on both stone fruit trees and the common reed (*P. communis*) (6). Consequently, ornamental stone fruit trees and weeds seem unlikely to become an important reservoir of the virus in this area. Hence, *P. cerasifera* probably does not have a role in the *PPV* epidemics in Ankara stone fruit gardens. However, it may be a reservoir for *PPV* in areas of fruit production where the virus has been eradicated. In the light of our results it can be concluded that the removal of rootstock suckers and the application of pesticides against aphids may be effective in the reduction of *PPV* infection. This could be economically beneficial for the orchards in the vicinity of ornamental stone fruits.

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