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Investigation of *Plum pox virus* in Different Tissues of Apricot and Plum Trees

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Abstract: In order to investigate *Plum pox virus* (PPV) in different tissues of infected apricot and plum trees, 50 samples per infected tree were collected from 5 apricot and 5 plum trees infected with PPV-M and D strains between 2002 and 2004. In addition, 25 apricot and 25 plum seeds taken from severely infected trees in order to investigation transmission of PPV through seeds. The detection of PPV was carried out using indirect ELISA. The virus was detected in bark, some flower parts (sepal, petal and stamen), pits (only in apricots) and fruits. However, the virus was not found in pollens, gynoeciums, seeds and seedlings. It is appears that pollens and seeds did not play role in natural spread of PPV in Ankara, Turkey.

Key words: *Plum pox virus*, detection, apricot, plum, flower, seed, fruit

INTRODUCTION

The *Plum pox virus* (PPV) is a member of the genus Potyvirus, which causes the economically important sharka disease on stone fruit trees. The disease was recorded for the first time in Bulgaria in 1918 on plum trees and then in 1933 on apricot trees. The disease was first reported on peach trees in 1961 in Hungary. Since then, the virus has progressively spread to the large portion of the Europe and around the Mediterranean basin (Nemeth, 1994). Based on the biological and molecular properties, PPV isolates can be divided into the following 4 groups: apricot isolate PPV-D (Dideron), peach isolate PPV-M (Markus) (Kerlan and Dunez, 1979), the Egyptian isolate PPV-EA (El Amar) (Wetzel *et al*., 1991) and cherry isolate PPV-C (cherry) (Nemchinov *et al*., 1996). The virus is non-persistently transmitted by a number of aphid species (Nemeth, 1994).

Symptoms of PPV vary considerably with the host plant species, cultivar, age and nutrient status of the plant, environmental conditions and the strains of the virus. PPV infects nearly all species of genus *Prunus*. Severe symptoms are observed in apricot, plum and peach trees. These symptoms appear on leaves, fruits, pits of fruits and flowers and include chlorotic pale green lines, rings and spots on apricot and plum leaves whereas the virus causes chlorotic blotches, rings and bands surrounding the veins. The infected fruit of apricots and plum trees show chlorotic rings, lines, reddish discolorations, deformations and irregular grooves. Clearcut rings and spots can be observed on the pits of apricots and plums although it causes white or green rings or spots on peach fruit without pit symptoms (Nemeth, 1986). Symptoms on flowers were only observed on petals of some peach cultivars in France (Desvignes, 1999).

The virus was first described in Turkey on plum trees in 1968 (Sahtiyanci, 1969). Today, PPV is widespread in home gardens in Ankara, but has limited distribution elsewhere in Turkey (Elibuyuk, 2003). PPV M and D strains exist in Turkey, but the EA strain has not been detected (Elibuyuk, 2004).

Since its identification in 1932, sharka disease has been studied extensively, often from different perspectives. Coman and Cociou (1976), Morvan and Castellain (1976), discovered the irregular localization of the PPV in infected trees. The presence and concentration of the virus in the infected plants vary considerably with plant cultivar, the age of plant and climatic conditions (Nemeth, 1986, Desvignes, 1999). Therefore, this study presents a detailed evaluation of the investigation of the *Plum pox virus* in different tissues of PPV-infected apricot and plum trees in Ankara conditions. Seed transmission of PPV might play an important role in the dissemination of this disease especially through breeding programmes, international exchange of germplasm and use of seedlings as rootstocks. The information available on the seed-borne transmission of PPV is controversial. Hence, the presence of PPV was also investigated in order to determine whether seeds and pollens play a role in the natural spread of this disease in Ankara.

MATERIALS AND METHODS

Bark, flower parts (sepal, petal, stamen, gynoecium and pollen), fruit, seed and leaf samples (as a control) were collected from PPV M and D infected apricot and plum trees (5 of each-unknown cultivars) in Ankara

between 2002 and 2004. Fifty samples per infected tree were collected which were then tested using indirect ELISA.

The indirect ELISA tests were completed using duplicate wells in polystyrene microtitre plates using the methods outlined by Uyemoto *et al.* (1989). Samples were extracted using a coating (carbonate) buffer (pH 9.6) containing 0.045 g L^{-1} sodium diethyl dithiocarbamate (Na-DIECA), 20 g L^{-1} polyvinyl pyrrolidone-25 (PVP) and 2 g L^{-1} bovine serum albumin (BSA), 1/20 w/v. Plates were incubated with plant extracts for 2 h at 37°C. The polyclonal PPV antiserum was diluted in a phosphate-buffered saline (PBS) that contained 20 g L^{-1} polyvinyl pyrrolidone-25 (PVP) and 2 g L^{-1} bovine serum albumin (BSA), 1/1000 v/v. The plates with the diluted antisera were loaded within 3 h and kept at 37°C. The alkaline phosphatase-conjugated goat anti-rabbit antiserum was later diluted in the latter buffer (1/50000 v/v) and the plates with conjugate were incubated for 3 h 37°C. The ELISA values were obtained by measuring the absorbance of the substrate at 405 nm, after incubation with an alkaline ρ-nitrophenyl phosphate in substrate buffer (0.5 mg mL^{-1}) at room temperature.

The investigation of seed-borne transmission of PPV involved the collection of apricot and plum seed taken from severely infected trees (25 seeds from each tree). The seeds were then stratified in perlite at 4°C (Stokes, 1965). After germination the seeds were transferred to the pots. Seedlings from stratified seeds were periodically tested for PPV infection using indirect ELISA over a 2-year period.

RESULTS AND DISCUSSION

Bark, flower parts (sepal, petal, stamen, gynoecium and pollen), fruit, seed and leaf samples were collected from PPV M and D infected apricot and plum trees and the following results were obtained. As well, the seedlings were evaluated for PPV infection.

Bark: Samples were taken in February, from the internodes and included the phloem and all surrounding tissue. PPV was detected in the samples of both the apricot and plum trees (Table 1). Adams (1978), Dosba *et al.* (1986), Adams *et al.* (1998) and Polak (1989) found similar results in studies that employed similar ELISA techniques. Adams (1978) reliably detected PPV using plum bark samples taken in January and February. According to the data obtained by Adams *et al.* (1998), PPV was detected in bark samples collected in December, January and March. Polak (1989) reported that PPV was reliably detected in bark of apricot trees. Thus, there is no contradiction between earlier investigations and the results of this study.

Flower: PPV was found in sepals, petals and stamens (Table 1). Petals are frequently used as a source of virus inoculum from woody plants but the study by Clark and Adams (1977) indicated that petals from *Prunus insititia* (damson plum) were a poor source of PPV compared with sepals and gynoeciums. A similar distribution of the virus was found in the flowers of *Prunus domestica* (plum) and *Prunus ceracifera* (myrobalan plum) (Adams, 1978). In contrast, Rankovic and Vuksanovic (1981) did not detect PPV in the petals of PPV-infected apricot trees.

In this study, PPV was also detected in stamens but was not detected in the pollen and gynoecia of the trees investigated (Table 1), a result supported by both Adams (1978) and Dosba *et al.* (1986). Trifonov (1965) first discovered the presence of the virus in the pollen of infected trees. Macovei (1970) pollinated healthy plum trees with the pollen of diseased plum cv. Agen then characteristic virus symptoms around the pollinated flowers the following year. Coman and Cociu (1976) found 20-80% pollen transmission according to the susceptibility of the plum cultivars. The above mentioned reports indicate that, there are documented cases of positive transmission of PPV by pollen. However it is generally thought that PPV is not transmits by pollen.

Fruit: PPV was detected in the fruit of infected apricot and plum trees (Table 1). Llacer *et al.* (1986) found that plum fruit was the most reliable parts of the plant for detecting PPV by ELISA. This is in agreement with the results obtained by van Oosten (1970) who used plum fruits for the mechanical transmission of PPV to herbaceous plants. Adams (1978) found that PPV could be detected on the fruit of plum trees. Similarly, Dosba *et al.* (1986) also detected PPV from on the fruit, bark, buds, flowers, stamens and leaves of apricot trees. Present results support these findings.

Table 1: Absorbance values of different tissues of the PPV infected stone fruit trees

Tree		Leaf	Bark	Sepal	Petal	Pollen	Gyno.	Stamen	Fruit	Pits	Seed
Apricot	H	0.151	0.141	0.157	0.145	0.145	0.148	0.160	0.176	0.155	0.143
	I	0.978	0.355	0.407	0.360	0.186	0.251	0.382	1.128	0. 405	0.152
Plum	H	0.156	0.148	0.161	0.157	0.154	0.153	0.168	0.185	0.142	0.138
	I	1.224	0.364	0.498	0.388	0.178	0.265	0.398	1.439	0.157	0.149

H: Healthy, I: Infected, Gyno.: Gynoecium

Pit: Pit rings were observed in many of the apricot fruits, whereas no symptoms were observed on plum pits. PPV was detected in apricot pits, but it was not detected on plum pits (Table 1). We found no literature regarding detection of PPV in pits.

Seed: In this study, PPV was not detected in the seeds (Table 1) and seedlings. Seed transmission in *Prunus* is not known to occur with the D strain, but there are some reports of seed transmission with the M strain. Seed transmission on apricots was first reported by Szirmai (1961). Coman and Cociu (1976) found 8-10% seed transmission depending on the susceptibility of the plum cultivars. Nemeth and Kölber (1982) found PPV in 23.2-79.4% of seeds originating from diseased apricot trees and reported 3.4-13.9% PPV infection in the 2- year-old seedling progeny of diseased trees. Eynard *et al.* (1991), Triolo *et al.* (1993) and Pasquini *et al.* (1998) detected PPV in apricot seeds; Glasa *et al.* (1999) found PPV in plum seeds and Pasquini *et al.* (2000) found PPV in apricot and peach seeds. However, seedlings from infected seeds have never showed symptoms and have been found to be PPV-negative by serological and molecular assays (Schimanski *et al.*, 1988; Eynard *et al.*, 1991; Triolo *et al.*, 1993; Dulic-Markovic and Rankovic, 1997; Myrta *et al.*, 1998; Pasquini *et al.*, 1998; Glasa *et al.*, 1999; Pasquini *et al.*, 2000). Most researchers think that the virus is inactive during seed germination. The most commonly held view of scientists working on PPV in many countries is, however, that seed and pollen transmission does not occur and the differences in results obtained are probably a result of the differentiated character of the various PPV strains (Myrta *et al.*, 1998; Desvignes, 1999; Pasquini *et al.*, 2000).

The apricot and plum seedlings were monitored for symptom expression over two years and tested using ELISA. Fortunately, none of the test specimens were ELISA positive showing no PPV symptoms. The virus could be detected on apricot pits (not plum pits) not the seeds indicating that PPV is not seed-transmitted.

In conclusion, bark tissue was found to be an opportune tissue in the detection of the PPV during the winter in Ankara. Despite the lack of antigen in some tissues, whole flowers are acceptable sources of antigen for ELISA. Some of the stone fruit varieties have no or less distinct symptoms on the fruit (Elibuyuk and Erdiller 1995) and the absorbance values of fruits increased as the symptom became more severe (Adams, 1978). Therefore the fruit of these trees is not a good source of PPV and thus not suitable for the detection of the virus. Further, the non-existence of PPV in pollen, seeds and seedlings is a reassuring result in terms of dissemination of the virus in Ankara as our results indicate that PPV was not transmitted via pollen or seeds in Ankara. We did find that the disease was transmitted by mealy plum aphids (*Hyalopterus pruni*) (Elibuyuk, 2003).

Our results indicate that PPV was detected consistently in extracts of most types of tissue tested using ELISA. However, because the distribution of the virus is uneven in infected trees (Nemeth, 1986), small numbers of samples from each tree may be inadequate to detect PPV. Therefore, a sufficiently large number of samples must be taken if ELISA assays are to be employed in the detection of this disease.

Our results were found to be similar to the found by other investigators. Nevertheless, the presence and concentration of the virus in the infected plants vary considerably with plant cultivar, the age of the plant and climatic conditions (Nemeth, 1986, Desvignes, 1999). Furthermore, because the distribution of PPV in infected trees is uneven, erratic results might be obtained in detection. Even though ELISA is a convenient detection method, molecular techniques (e.g., RT-PCR) has improved PPV diagnosis, as these techniques are more sensitive than serological tests and can detect very low virus concentrations in plant tissues (Wetzel *et al.*, 1992). However, even if PPV is found in pollen and seeds, seed and pollen-transmission studies should still be performed.

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