SHORT COMMUNICATION

OCCURRENCE AND DISTRIBUTION OF STONE FRUIT VIRUSES AND VIROIDS IN COMMERCIAL PLANTINGS OF *PRUNUS* SPECIES IN WESTERN ANATOLIA, TURKEY

M. Gümüs[1], I.C. Paylan[1], S. Matic[3], A. Myrta[2], H.M. Sipahioglu[4] and S. Erkan[1]

[1] *Department of Plant Protection, Faculty of Agriculture, Ege University, 35100 Bornova, Izmir, Turkey*
[2] *Istituto Agronomico Mediterraneo, Via Ceglie 9, 70010 Valenzano (BA), Italy*
[3] *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy*
[4] *Department of Plant Protection, Faculty of Agriculture, University of Yuzuncu Yil, 65080 Van, Turkey*

SUMMARY

Symptoms of virus and viroid infections have been observed during the last ten years in stone fruit crops, however, no relative incidences of virus and viroid diseases have been reported on stone fruit crops in western Anatolia, Turkey. Large-scale surveys were conducted from June to August between the years 2004 and 2006 in the main stone fruit growing orchards and mother blocks of western Anatolia to determine the seven most important virus and two important viroid affecting *Prunus* species. The results of serological (ELISA) and molecular (PCR and tissue-printing molecular hybridization) tests demonstrated the occurrence of *Plum pox virus* (PPV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Apricot latent virus* (ApLV), Plum bark necrosis stem pitting-associated virus (PBNSPaV), *Peach latent mosaic viroid* (PLMVd), and *Hop stunt viroid* (HSVd) in tested 1732 specimens of stone fruits. The overall infection level with these graft-transmissible agents was 30%. The results showed that PDV is predominant in stone fruit crops. To our knowledge, PBNSPaV was reported for the first time in western Anatolia. The results obtained in this study illustrate a need for certification or clean stock program to prevent the occurrence and the spread of graft transmissible agents in western Anatolia.

Key words: *Prunus* viruses and viroids, stone fruits, diagnosis, RT-PCR, Turkey.

Stone fruits are an important crop in most parts of the world. Turkey is one of the most important stone fruit suppliers as it produces around 1.3 million tons annually (FAOSTAT, 2005). Bursa, Canakkale, Izmir, Aydin, Afyon, Kutahya and Manisa (western Anatolia) are the main stone fruit-producing provinces of Turkey. Stone fruits are known to be susceptible to many virus and viroid diseases and can be infected with more than 25 graft-transmissible diseases (Myrta *et al*., 2003). Among them, important pathogens include: *Plum pox virus* (PPV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV), *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Apricot latent virus* (ApLV), Plum bark necrosis stem pitting associated virus (PBNSPaV), *Peach latent mosaic viroid* (PLMVd), and *Hop stunt viroid* (HSVd) (Abou Ghanem-Sabanadzovic *et al*., 2001; Myrta *et al*.; 2003; Bouani *et al*., 2004).

In previous studies, PPV, PDV, PNRSV, ACLSV, ApMV, ApLV, PBNSPaV, PLMVd, and HSVd were found with differing incidences (0.3 - 77%) in regions of Turkey (Elibuyuk and Erdiller, 1991; Baloglu *et al*., 1995; Caglayan and Hurigil, 1996; Sipahioglu *et al*., 1999; Gümüs *et al*., 2004; Ulubas and Ertunc, 2005; Sipahioglu *et al*., 2006; Usta *et al*., 2006). Only PBNSPaV has not yet been found in western Anatolia.

In recent years, virus diseases have become a limiting factor in the production of stone fruits in western provinces of Turkey. Tissue chlorosis, chlorotic spots, mottle, and/or mosaic symptoms on leaves, leaf distortions, stunting of the plant, severe bark cracks, and stem pitting on tree trunks are symptoms commonly observed on fruit trees. There are several reports of the presence of virus and viroid diseases of stone fruits in western Anatolia, but no information on their incidence. The present investigation was undertaken to secure this information.

Leaf samples were collected in the growing seasons of 2004, 2005 and 2006 from plants in peach, apricot, cherry and plum orchards and mother blocks that showed stunting, chlorotic spots and/or mosaic, vein banding, enation, or shot-hole symptoms. The surveyed regions were in seven major regions of western Anatolia: Bursa, Canakkale, Izmir, Aydin, Afyon, Kutahya and Manisa provinces. In total, 1732 stone fruit trees were sampled.

A total of 1225 samples of *Prunus* species were assayed for the presence of PDV, PNRSV, PPV, ACLSV, and ApMV by ELISA (Clark and Adams, 1977), using

Corresponding author: M. Gumus
Fax: +90.23.23744848
E-mail: mustafa.gumus@ege.edu.tr

antisera from Bioreba AG (Reinach, Switzerland). Virus-free peach seedlings (*Prunus persica* GF 305) grown in an insect-proof growth chamber were used as negative controls. Samples were considered to be positive when the absorbance values at 405 nm (A_{405}) values exceeded the mean of the negative controls by least a factor of two.

RT-PCR was used to assay 155 samples. Total RNA was extracted from plant tissue using a silica capture method (Boom *et al*., 1990) and reverse transcription was performed using a cDNA synthesis kit (MBI Fermentas, GmbH, St. Leon-Rot, Germany). PCR of 120 apricot samples for ApLV was as described by Nemchinov and Hadidi (1998) and PCR of 35 samples of cherry, plum or peach for PBNSPaV was done using a nested RT-PCR protocol (Abou Ghanem-Sabanadzovic *et al.,* 2001; Amenduni *et al.*, 2004). A field-grown apricot (P*runus armeniaca* L.) isolate of ApLV and a sweet cherry (*Prunus avium* L.) isolate of PBNSPaV identified from preliminary tests from the Izmir province, were used as positive sources for diagnosis of ApLV and PBNSPaV. Leaf tissues of one-year-old shoots of infected apricot and sweet cherry were used in the RT-PCR assays. Amplified products were separated by gel electrophoresis in 1.5% agarose gel in 40 mM Tris pH 7.8, 20 mM acetic acid, 2 mM EDTA and DNA was detected after staining with ethidium bromide.

Tissue print molecular hybridization was used to assay 352 samples for PLMVd and HSVd as described by Shamloul *et al*. (1995) and Pallas *et al*. (1997), respectively. RNA was extracted as described by Astruc *et al*. (1996) and hybridization assays were done on positively charged Hybond N+ membrane. Nucleic acids were cross-linked with UV light, then membranes were subjected to molecular hybridisation using specific digoxigenin-labeled full-length cRNA riboprobes (Ambros *et al*., 1995). Chemiluminescent detection, using Fab-fragments conjugated to alkaline phosphatase, was according to the manufacturer's (Boeringher, Mannheim, Germany) recommendation.

In surveyed orchards, over 20,000 fruit trees were inspected for virus and viroid symptoms. Symptomatic trees showing chlorotic spots and malformed fruits associated with PPV, mottle, mosaic, vein banding, shot holes on leaves, stem pitting on tree trunks associated with PBNSPaV. Color break in peach petals in the spring were observed in many stone fruit orchards.

Serological tests showed that stone fruit trees were infected with ACLSV, PPV, ApMV, PNRSV, and PDV. Of the 1225 stone fruit samples tested, 365 were infected with at least one of the investigated viruses. PDV was the most common with a incidence of 14%. Infection frequencies for ACLSV, PNRSV, ApMV, and PPV were 4%, 6%, 0.8%, and 0.4%, respectively. The percentage of infected species was 35%, 31%, 26%, and 8% for cherry, peach, plum and apricot, respectively. The infection rate was highest in Kutahya (67%) and lowest in Manisa (11%).

In general, apricot orchards appeared to be relatively healthy. Of the 120 apricot samples tested, 19 were positive for ApLV in RT-PCR. All infected samples yielded the expected amplicon of 200 bp (Nemchinov and Hadidi, 1998). The virus was detected in the cvs Proyma and Igdir in Izmir province. The relative incidence of ApLV (15%) in western Anatolia was higher than that reported (1.3%) for eastern Anatolia (Usta *et al*., 2006). This could be due to the higher circulation rate of planting materials in the west. ApLV has been recorded from different countries where apricot is grown, i.e. Moldova and Bulgaria (Zemtchik and Verderevskaya, 1993; Zemtchik *et*

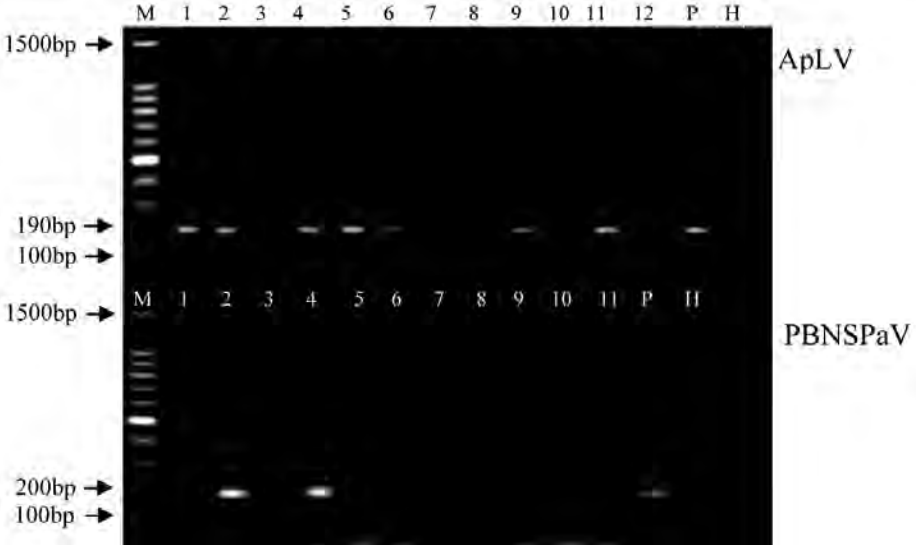

Fig. 1. ApLV and PBNSPaV positive samples detected by RT-PCR and nested-RT-PCR respectively. M is molecular size markers; P is positive virus control; H is healthy control.

al., 1998), France and Italy (Gentit *et al*., 2001) and more recently in Palestine (Abou Ghanem-Sabanadzovic *et al*., 2005) and Turkey (Gümüs *et al*., 2004).

Of the 35 stone fruit samples (10 of peach, 12 of plum, and 13 of cherry) tested by nested-RT-PCR, only two cherries were positive for PBNSPaV as they yielded a DNA fragment of 190 bp in length with primers ASPn1/ASPn2 developed by Abou Ghanem-Sabanadzovic *et al.* (2001) (Fig. 1). This is the first record of PBNSPaV from western Anatolia. Although surveys for PBNSPaV were limited compared to other viruses, a few pitted cherry and plum trees were observed in Izmir province. PBNSPaV was characterized only recently (Abou Ghanem-Sabanadzovic *et al*., 2001; Amenduni *et al*., 2004) and there are limited reports on the distribution of the virus in the world. Usta *et al*. (2006) reported the presence of the virus in cherry, plum and prune trees in eastern Anatolia with an incidence of 77%.

Among 352 stone fruit samples, 88 were found to be infected by viroids. Of these, 61 peaches and one apricot were infected by PLMVd. The remaining 26 samples of apricot, peach or plum were infected by HSVd. The percentages of infected stone fruit plants were 17% for PLMVd and 7% for HSVd. Fifty samples were found to have mix infections, of these 48 were double and two were triple infections. PDV+ACLSV was the most frequently detected mixed infection. ELISA tests showed that two samples were infected with PDV+ PNRSV+ACLSV. Two mixed infections of PLMVd+ HSVd were recorded among viroid-infected samples, one in peach and one in apricot. The incidence of viroids in western Anatolia (25%) is much greater than the 3% in eastern Anatolia reported by Sipahioglu *et al*., (2006). However, this infection level was lower than that in other countries: 82% in Spain (Badenes and Llacer 1998); 62% in Syria (Ismaeli *et al*., 2001); 52% in Albania (Torres *et al*., 2003); and 50% in the USA (Skrzeckowski *et al*., 1996).

The present survey provides a relatively clear picture of the sanitary status of stone fruits crops. PBNSPaV was detected for the first time in western Anatolia, in addition to ACLSV, PDV, PNRSV, ApMV, PPV, ApLV, PLMVd and HSVd, that had been reported previously.

The incidence of virus and viroid differed, which could be attributed to the initial introduction of virus- and/or viroid-contaminated plants that were grown for many years in orchards, allowing dissemination. PDV was the most frequently found virus throughout the stone fruit growing area of western Anatolia, perhaps because PDV infects hosts other than cherries.

The aphid-transmitted PPV was the least frequent virus in plum and apricot and was not detected at all in peach and cherry, while ApMV, PNRSV and ACLSV were relatively frequent in the tested species. Azeri (1994) found PPV in only 0.4% of peach trees in western Anatolia. Yurekturk (1984) reported PPV, ACLSV and some ilarviruses in peach trees in the Marmara region, but surveys made in different sites of Turkey, did not find PPV infections (Elibuyuk and Erdiller, 1991; Baloglu *et al*., 1995; Caglayan and Hurigil 1996, Sipahioglu *et al*., 1999, Ulubas and Ertunc, 2005). Multiple infected plants showed more severe leaf symptoms and yield losses in our study than plants infected with just one of the viruses, probably due to a synergistic effect, as reported by Nemeth (1986) and Dunez (1988).

The serological and molecular tests performed in our study have secured information on the relative incidence of viruses and viroids infecting stone fruits, whereas previous investigations were primarily focused on the occurrence of diseases in the field and did not provide data on the relative occurrence of viruses in the different provinces of the region.

Some of the viruses detected in the current study are spread either by aphids, grafting, pollen or seeds (Nemeth, 1986). Since orchard owners in the region are little aware of how viruses and viroids spread and how their dissemiation can be controlled, we are confident that the results of this survey will be useful for the establishment of virus-free certification or clean stock programmes in western Anatolia.

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