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DETECTION AND IDENTIFICATION OF PLUM POX VIRUS ON PRUNUS SPECIES IN CRIMEA

SUMMARY

Plum pox virus (PPV, genus *Potyvirus*, family Potiviridae) is the causal agent of the Sharka disease and considered the most detrimental viral pathogen of stone fruits. PPV spread over long distances due to uncontrolled exchanges of infected plant material. The spread of Sharka virus (*Plum pox virus*) in different regions of the Crimea was obtained by monitoring of its viral pathogen in commercial orchards and collection plots of *Prunus persica*, *P. persica* var. *nectarine*, *P. armeniaca*, *P. cerasifera*, *P. domestica*, *P. avium*. Different symptoms of the disease on flowers, leaves and fruits caused by *Plum pox virus* were revealed. Using of complex diagnostic methods (ELISA-test and PCR-analysis) allowed us to identify the viral pathogen mostly in peach, nectarine and myrobalan plum plant material. The results of RT-PCR analysis indicate that all the isolates from peach, nectarine, plum, myrobalan plum cultivars tested belong to the PPV-D strain.

Key words: Plum pox virus, stone fruits, monitoring, symptoms, isolates, strain

INTRODUCTION

Steppe and subtropical regions of the Crimean peninsula are exclusively favorable for the cultivation of stone fruits that occupy significant cultivation areas and play an important role in the peninsula economics. Also, there is an unique stone fruit germplasm field collection in Nikita Botanical Gardens (Yalta, Crimea), represented by many local and introduced cultivars and hybrid forms of peach [*Prunus persica* L. (Batsch)], nectarine [*P. persica* var. nectarine (Aiton) Maxim], apricot (*P. armeniaca* L.), plum (*P. domestica* L.), myrobalan plum (*P. cerasifera* Ehrh.), sweet cherry (*P. avium* L.). *Prunus* plants are known to be possibly infected by many viruses (Mitrofanova et al., 2000; Herranz et al., 2005; Pallas et al., 2012). However, the plum pox potyvirus (*Plum pox virus*, PPV, genus *Potyvirus*, family Potyviridae) is considered the most detrimental viral pathogen of stone fruits affecting their yield and fruit quality (Cambra et al., 2006). The first PPV symptoms we observed in the Crimean orchards in 1994 (Mitrofanova et al., 1997).

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PPV is a causal agent of Sharka disease. Under natural conditions and experimentally PPV is able to infect the *Prunus* spp. and a number of herbaceous species from other taxa (Llacer and Cambra, 2006; James and Thompson, 2006; Llacer, 2006). From plant to plant PPV may be transmitted by vegetative propagation and aphids in a non-persistent manner. Over long distances, PPV is spread through the exchanges of infected plants. Sharka disease is distributed worldwide with the exception of Australia, New Zeeland, South Africa and California. Based on genomic differences, nine PPV strains are recognized to date: Dideron (D), Marcus (M), Recombinant (Rec), Cherry (C), Cherry Russian (CR), El Amar (EA), Winona (W), Turkish (T) и Ancestral (An). The strains differ in antigenic and epidemiological properties, host range, geographical distribution and pathogenicity for stone fruit species. The strains D, M and Rec are widespread in Europe and Mediterranean region. The strains C, CR и W, with few exceptions, were only found in the former USSR until now (Garcia et al., 2014).

In this paper we present the results of PPV monitoring in the stone fruits orchards in different climatic areas of the Crimean peninsula aimed to PPV detection and identification.

MATERIAL AND METHODS

The suspected buds, flowers, leaves and fruits were sampled in commercial stone fruit orchards and surrounding wild plantings in Yaltinskii, Bahchisaraiskii, Sevastopolskii, Simferopolskii, Sakskii, Krasnogvardeiskii and Dzhankoiskii districts of the Crimea and also in the Prunus collection plot of Nikita Botanical Gardens. The PPV infection was confirmed by double antibody sandwich (DAS) ELISA using a reagent sets SRA 31505 (Agdia, USA) and Pyrotest kits (Immunotek, Russia) according to the supplier's protocols. Immunocapture RT-PCR was performed according to Wetzel et al. (1992) using polyclonal PPV-specific antibodies (Agdia, USA), oligo-dT primer for the first strand cDNA synthesis and primers specific for D, M, C, CR, W and Rec strains, according to the original protocols (Olmos et al., 1997; Nemchinov and Hadidi, 1997; James and Varga, 2004; Subr et al., 2004; Glasa et al., 2013).

The investigations were carried out in the Plant Biotechnology and Virology Laboratory of Plant Developmental Biology, Biotechnology and Biosafety Department at Nikita Botanical Gardens.

RESULTS AND DISCUSSION

Monitoring of *Plum pox virus* (PPV) has been carried out in stone fruit collection plots and orchards in different regions of the Crimea. PPV distribution and identification of the main diagnostic features of the disease were conducted from March to October 2014 and from March to June 2015. It has been found out that appearance of visual disease symptoms coincided with such phenologycal phases of plant development as flower buds coloration, flowering, leaf and fruit development, that let us to determine optimal terms for collecting samples –

April - June and August - September. During the investigation general state of plant and PPV symptoms occurrence on flower buds, flowers, leaves and fruits were estimated.

First PPV symptoms were observed on flower buds and petals of peach, nectarine, myrobalan plum and plum in April - May (Fig. 1). In Yaltinskii and Bahchisaraiskii districts for peach cultivars Granatovyi, Dostoinyi, Zolotaya Mosckva, Early Red Haven and nectarine cvs. Maria Marta, California, Venus, Caldesi crimson lines and spots were noticed on the petals. In myrobalan plum cvs. Purpurnaya, Obilnaya and plum cvs. Izum Erik, Kleimen, Renclod Altana PPV symptoms include spot necrosis and petals deformation. In Simferopolskii district rings and bands were clearly observed on the flowers of peach, myrobalan plum and plum. In Krasnogvardeiskii district on peach and myrobalan plum plants some other PPV symptoms were noticed – dark line patterns and small spot necrosis. In plants with PPV symptoms the deformation of flower buds and petals was often observed.

Typical PPV symptoms on leaves and fruits were noticed in May - September: on leaves – May - August, on fruits – June - September (Fig. 2 and Fig. 3). On the leaves of peach and nectarine plants PPV symptoms include chlorotic spots, rings, bands, halos. Leaves may be distorted, twisted and banded along the central vein. Such symptoms were noticed on the leaves of peach cvs. Beketovsky, Garmonia, Granatovyi, Dostoinyi, Zolotaya Mosckva, Pontijskii, Slava Stevena, Tulpan, Early Red Haven and nectarine cvs. Flavor Gold, Maria Marta, May Grand, California, Caldesi. Occurrence frequency of these symptoms differed due to the region of plants` growth. So, in Yaltinskii district on peach and nectarine plants such disease symptoms as rings, bands and halos gave the part from 17 to 40%, chlorotic spots – from 16.6 to 24.5%, distorted and twisted leaves – 12.6-21.4%. Though on the leaves of apricot cvs. Detskii, Limonno-Zheltyi, Markuleshti, Mechta, Mandule Kayszi yellow-green rings, spots and bands were observed. Similar symptoms were noticed for myrobalan plum cvs. Altsina, Bordovaya, Purpurnaya. In plum cvs. Izum Erik, Kleimen, Pop Hariton, Renclod Altana, Stenley, and Verity little chlorotic spots, rings, bands and lines were found.

A B

Figure 1. PPV symptoms on the flowers of nectarine (A) and peach (B)

A B

C D

Figure 2. PPV symptoms on the leaves of nectarine (A, B), plum (C) and peach (D)

On the immature fruits of peach and nectarine chlorotic spots and light rings were observed. On mature fruits red rings, bright purple-red spots or large halos with pink centre were found in cvs. Sunbeam, Trakijskii Rannii, Red Haven and etc. In highly damaged cultivars fruit stones were deformed. On apricot fruits cvs. Detskii, Limonno-Zheltyi and Markuleshti discolored spots with green or carmine ring and tuberosity were noticed. In apricot cultivar Limonno-Zheltyi violet rings on the immature fruits were observed. In damaged fruits of cv. Markuleshti rings and spots were observed on the stones. In plum cvs. Izum Erik, Pop Hariton light halos with darker centre and sunken lesions occurred, fruits were deformed and stones had dark spots. It has been found out that in the studied cultivars PPV damaged fruits ripen irregularly and drops that leads to the yield loss and poorer production quality.

A B

C

Figure 3. Sharka symptoms on the fruits of nectarine (A), peach (B) and apricot (C)

Use of various diagnostic methods helped to find and identify PPV in the collected samples. In total, 4925 samples of peach, nectarine, plum, myrobalan plum and apricot representing more than 2000 cultivars and hybrid forms of stone fruit plants were collected and studied for the supposed PPV infection by ELISA tests. The data on PPV incidence is summarized in Table 1.

Table 1. Incidence of *Plum pox virus* in *Prunus* species in different regions of the Crimea

	Number of *Prunus* samples, tested/infected (%)		
Region	Peach and nectarine	Myrobalan plum and plum	Apricot
Yaltinskii	500/38.2	270/37.5	203/10.0
Sevastopolskii	350/46.4	179/54.7	120/11.3
Bahchisaraiskii	1135/40.2	270/9.7	nt*
Simferopolskii	300/7.6	185/13.1	240/9.2
Krasnogvardeiskii	300/12.8	198/11.4	nt
Sakskii	250/11.2	nt	nt
Dzhankoiskii	200/7.9	120/5.1	105/3.8

"nt" – not tested

Among 500 tested samples of peach and nectarine taken in Yaltinskii and Sevastopolskii districts presence of PPV was proved for 38.2 and 46.4%, respectively. Among 197 apricot samples PPV was found in 21.3%. Among 270 myrobalan plum samples 37.5% had positive reaction. The highest percent of damaged samples (54.7%) was determined for plum in Sevastopolskii district. Among 1135 tested nectarine and peach samples collected in Bahchisaraiskii district PPV was identified in 40.2%. Since some nectarine cultivars were strongly damaged with PPV (+++): California (81.9%), Caldesi (66.9%), Maria Marta (57.9%). Through 270 plum samples PPV was found in 9.7%. Samples taken in the central part of the Crimea were less damaged. Thus, among 600 tested peach samples collected in Simferopolskii and Krasnogvardeiskii districts PPV was found in 7.6 and 12.8%, respectively; through 240 apricot samples it was determined in 9.2% and among 383 tested plum samples PPV was identified in 13.1 and 11.4%, respectively. In Sakskii and Dzhankoiskii districts among 450 peach samples PPV was identified in 11.2 and 7.91% of samples, respectively, through 120 tested myrobalan plum samples PPV was found in 5.1%, through 105 apricot samples it was determined in 3.8% (Table 1).

The data presented indicate for the first time a widespread of PPV in stone fruit commercial and collection plantings in the Crimea. They also demonstrate that PPV is more frequently occurred in subtropical area than in temperate steppe areas.

At the same time, strain identification is very important for understanding of the virus evolution and has a great practical significance. The strain of PPV isolates found on peach, nectarine, plum and myrobalan plum in Nikita Botanical Gardens'

collection plots was performed using RT-PCR. The 198 bp products were obtained by RT-PCR analysis using the PPV-D-specific P1/PD primers (Fig. 4). No reaction with primers specific to other PPV strains was observed (data are not presented). These results indicate that all the isolates tested belong to the PPV-D strain. The PPV-D strain is widespread in Europe and probably is the most distributed in the world (Maejima et al., 2011; James et al., 2013; Garcia et al., 2014). The origin of the PPV-D isolates in Crimea as well as their phylogenetic relationships has to be elucidated yet.

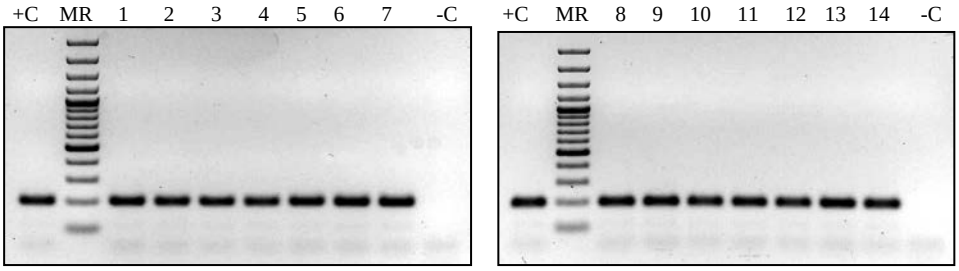

Figure 4. Agarose gel electrophoresis analysis of reverse-transcription polymerase chain reaction amplification products from PPV-infected leaf samples using P1/PD primers.

MR - GeneRuler 100 bp DNA ladder (Fermentas). The arrows on the right show the products specific for the PPV-D (198 bp). Samples from peach: cvs. Zolotaya Moskva (1), Slava Stevena (2), Tulpan (3), Dostoinyi (4), Sunbeam (5), Favorita Morettini (6), Red Cup (7), hybrid form 83-878; nectarine: cvs. May Grand (8), Flavor Gold (9), hybrid 594-81 (10); plum: cvs. Kleimen (11), Izum Erik (12); myrobalan plum cv. Purpurnaya (13), wild myrobalan plum (14). "+C" and "–C" – positive and negative controls, respectively (Agritest, Italy)

CONCLUSIONS

Plum pox virus monitoring in the Crimea`s stone fruits commercial orchards and collection plots has demonstrated a high rate of cultivar damages depending on the district of growing. Main diagnostic features of virus infection occurrence on flowers, leaves and fruits have been found out and optimal terms for collecting samples of peach, nectarine, apricot, plum and myrobalan plum cultivars (April - June, August - September) have been established. The greatest damage of stone fruit cultures was noticed in the southern and central regions of the Crimea.

The PPV-D strain was identified in the Crimean region. Using of complex analyzing methods gave us possibility to determine the main areas affected by PPV and to recommend the infected plants elimination. Further application of biotechnology methods will promote the obtaining of PPV-free plants.

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