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TULPINI DE PLUM POX VIRUS IDENTIFICATE LA PIERSIC IN ZONA CENTRAL-SUDICĂ A BULGARIEI

PLUM POX VIRUS STRAINS IDENTIFIED IN PEACH IN SOUTH CENTRAL REGION OF BULGARIA

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Abstract

Sharka disease, caused by plum pox virus (PPV), was detected on peach (*Prunus persica* (L.) Batsch.) for the first time in Bulgaria in 1989. Nowadays PPV is widespread in peach crops grown under varied agro-ecological conditions. The current study aimed to determinate the PPV strains spread in peach in six agro-ecological micro-regions located in three provinces (Plovdiv, Pazardzhik, Stara Zagora) of South Bulgaria. Altogether 70 PPV accessions, collected from 23 peach cultivars, were analysed by reverse transcription – polymerase chain reaction (RT-PCR) using primer pairs discriminating isolates of PPV-M, PPV-D and PPV-Rec strains. The summarized results revealed that PPV-M and PPV-D were identified in 85.7% and 8.6 % of the analysed samples, respectively. Both strains were detected at the highest infection rates in Pazardzhik province. Co-infection by both strains PPV-M and PPV-D was registered at the rate of 5.7 % of the tested accessions. PPV-Rec strain was not identified neither single nor in co-infection with the other strains. It has found differences in strain status in each of the studied orchards located in one and the same province. Likely the continuous increase of the PPV infection rate in peach is due to the prevalence of PPV-M strain, considered the epidemic form of the virus, and possible arising of higher virulent PPV-D variants. In that case, it should be applied all reasonable measures for containment of the virus spread and protection of the tree health.

Cuvinte cheie: boala Sharka, plum pox Virus, tulpini, piersic.
Key words: Sharka disease, plum pox virus, strain prevalence, peach.

1. Introduction

Plum pox virus (PPV), the causative agent of Sharka disease, is one of the most harmful pests on stone fruits world-wide. Scholthof et al. (2011) listed PPV to a group of 'Top 10' plant viruses in plant virology because of the efforts on its molecular research and economic impact on stone fruits production. For the first time the virus was discovered on plum in Bulgaria by Atanasoff (1933). Years after the first report, the Bulgarian research stated contradictory stand on natural susceptibility of peach as host of PPV till 1989 when it was proved undoubtedly that PPV infects peach under the field conditions of Bulgaria (Yankulova et al., 1990).

The most frequent observed Sharka symptoms on the peach leaves are vein clearing, swelling, distortion, small chlorotic rings and bands. On the peach fruits, infected by PPV, could be noticed colour break, rings, in some cases deformation and deterioration of fruit quality. In susceptible peach genotypes Sharka disease could destroys seriously the infected trees.

In the last 15 years it has been observed an increase of PPV infection rate and severe fruit symptoms in peach and nectarine. The results from serological testing published in 1997 (Topchiiska, 1997) showed an infection rate of 15.7% in peach. About ten years later an infection rate of 21% in Plovdiv region was recorded (Milusheva & Kamenova, 2006). According to the latest reported survey, carried out during 2009-2016, PPV infection rate of 40 % was found in peach (Kamenova & Borisova, 2019). Our current observations carried out by visual inspections and testing by enzyme-linked immunosorbent assay (ELISA), registered PPV infection rate of approximately 60% in the inspected peach orchards in South Bulgaria (Milusheva, unpublished data).

To date, ten PPV strains - D, M, Rec, C, EA, W, T, CR, An (James et al. 2013) and CV (Chirkov et al., 2018) are recognized on the base of the differences in genome sequences and phylogenetic analysis. So far in Bulgaria the PPV-M, PPV-D and PPV-Rec strains have been identified on stone fruits and in particular in peach (Kamenova & Borisova, 2019).

Because of the continued increase of PPV infection rate in peach the current research aimed to determinate PPV strains occurred in peach, grown in six micro-regions, located in Plovdiv, Pazardzhik and Stara Zagora provinces, situated in South Bulgaria.

2. Material and methods

Origin of the plant material and preselecting test

The survey was carried out during 2021-2023. One hundred and sixteen samples for the survey were collected from fourteen freestone, three clingstone (*Prunus persica* (L.) Batsch.), and six nectarine (*Prunus persica* var. *nectarine*) cultivars, grown in six micro-regions located in Plovdiv, Pazardzhik and Stara Zagora provinces (Fig. 1). The samples were collected from trees manifesting typical Sharka symptoms. Samples from asymptomatic surrounded trees were taken too.

A preselecting test by enzyme linked immune sorbent assay (ELISA) for general detection of PPV was performed according to Clark & Adams (1977), using commercial antiserums supplied by Agdia-EMEA. Than 70 ELISA PPV positive samples were subjected for further analysis by two step reverse transcription (RT) – polymerase chain reaction (PCR). The number, origin and host-cultivar of the preselected PPV infected peach trees are presented in Table 1.

Two steps reverse transcription (RT) – polymerase chain reaction (PCR)

RT-PCR was carried out for strain identification of the three major PPV strains, reported in peach. The strain - specific RT-PCR tests were performed with the primer pairs mM5/mM3, mD5/mD3 and mD5/mM3 (Subr et al., 2004) distinguishing PPV-M, PPV-D and PPV-Rec isolate, respectively, in the genomic region corresponding to C-terminus of the viral replicase and N-terminus of the coat protein. Total RNA was extracted by Spectrum Plant Total RNA kit (Sigma Aldrich, USA). RT step for synthesis of complementary DNA (cDNA) was performed with SCRIPT cDNA Synthesis kit (Jena Bioscience, Germany) according to instructions of manufacturer using random hexamer. Subsequent PCR of the cDNA was implemented with 2x Ruby Hot Start Master (Jena Bioscience, Germany). The PCR products obtained were separated by agarose gel electrophoresis and visualized under UV light.

3. Results and discussions

The summarized results from strain - specific RT-PCR showed that PPV-M and PPV-D were identified in 85.7% and 8.6 % of the analysed samples, respectively (Table 2). Mixed infection by both strains PPV-M and PPV-D was registered at the rate of 5.7 % of the tested accessions. At the highest rate of 90% PPV-M strain was detected Pazardzhik province. PPV-D was registered at the same rate (10%) in the samples from Plovdiv and Pazardzhik provinces. Mixed infection by both strains was found at higher rate in the samples from Stara Zagora province but it was not registered in the samples originated from Pazardzhik province. It has found differences in strain status in each of the studied orchards located in one and the same province. In Plovdiv province PPV-M and PPV-D was identified in both studied locations while in Pazardzhik and Stara Zagora provinces PPV-D was detected by one of the surveyed locations. PPV-Rec was not identified neither single, nor in co-infection with the other strains.

In all PPV positive tested cultivars symptoms on the fruits were observed, more pronounced in the trees infected by PPV-M or co-infected by both strains than those single infected by PPV-D (Fig. 2).

Among the PPV strains, the most frequently detected strain PPV-M is considered the epidemic form of the virus. It is assessed with wider host range and higher epidemic potential than other strains, preconditioned on its stronger virulence and aggressiveness. Also, its ability to be transmitted more effectively by aphid vectors, contributing its faster spread in orchards. That is one of the possible reasons for the increase of PPV incidence in peach orchards during the last years. PPV-D is a milder strain, spreads slower, especially under conditions of high infection pressure by PPV-M. The main hosts of PPV-D are plum and apricot and it is more difficult to infect peach (James, 2017). PPV-D was detected in separate orchards situated in the three surveyed provinces although Kamenova & Borisova (2019) have not identified this strain in peach from South Bulgaria. However, a higher infection rate of PPV-D was recorded in peach compared to the infection rate reported in our large-scale previous study from 2005-2008 (Milusheva, 2008). In comparison, the PPV-D infection rate then recorded in peach was 3.5%, while in the present study PPV-D was identified in 8.57% of the tested samples. That data shows that the rate of PPV-D infection in peach over the past 15 years, although slightly, has increased in areas dominated by PPV-M. The probable reason for this trend is that there has been a change in the population of PPV-D under the influence of environmental factors or due to the introduction of new strain variants with the import of cultivars and rootstocks of stone fruit species. In this study PPV-D was identified in four of six micro-regions. Likely PPV-D is spread in separate orchards where has been introduced probably through the planting material.

In spite of PPV-Rec was reported in peach in Southwest Bulgaria, (Kamenova & Borisova, 2019, Kamenova et al., 2011,) the strain was not detected in the studied location. Similar to PPV-D, PPV-Rec also difficult infects peach.

4. Conclusion

Prevalence of PPV-M in peach orchards and possible arising of PPV-D variants with higher virulence are the probable reason for the continuously increase of the PPV infection rate in peach in the last years. If the distribution of PPV continues with the same speed there exists real risk of early decline of the peach trees and destruction of orchards as all of that leads to negative ecological and economical effects. To contain further PPV spread in peach and other stone fruits strong measures for control to be applying. Usage of healthy planting material, distance from old stone fruit orchards, early virus detection, and control of the aphid vectors are fundamental preconditions for protection of the tree health.

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Tables and Figures

Table 1. Data on the locality of the surveyed orchards, host-cultivar and number of tested trees

Province/Location/ Coordinates	Cultivar	Number of tested samples
Plovdiv/Ostromila 42° 6′ 48.5″ N, 24° 43′ 40.74″ E	***Freestone peach***	
	Glohaven	2
	Elite 17-98	1
	Puldin	2
	Damianka	2
	Nectarine	
	Adriana	2
	Gergana	3
	Clingstone peach	
	Lamone	1
Plovdiv/Bolyantsi 42° 2′ 26.59″ N, 24° 56′ 52.99″ E	***Freestone peach***	
	Maycrest	2
	July Lady	1
	Suncrest	2
	Lucie	4
	Evmolpiya	2
	Nectarine	
	Fantasia	2
	Seegold	1
	Nectagrand	1
	Clingstone peach	
	Baladen	1
	Maria Serena	1
Pazardzhik/Byaga 42° 4′ 0.11″ N, 24° 22′ 44.29″ E	***Freestone peach***	
	Redhaven	5
	Cresthaven	4
	Nectarine	
	Fantasia	3
Pazardzhik/Dragor 42° 13′ 39.51″ N, 24° 18′ 11.6″ E	***Freestone peach***	
	Redhaven	4
	Elegant Lady	4
Stara Zagora/Cherganovo 42° 35′ 5.8″ N, 25° 28′ 11.94″ E	***Freestone peach***	
	Redhaven	6
Stara Zagora/Vetren 42° 36′ 23.5″ N, 25° 41′ 27.21″ E	***Freestone peach***	
	Symphony	7
	Tardi bell	3
	Nectarine	
	Orion	4
Total		70

Table 2. Infection rates of PPV strains identified in the surveyed locations and province

Provinces/Location	PPV-M		PPV-D		PPVM+ PPV-D	
	n/N	%	n/N	%	n/N	%
Plovdiv/Ostromila	11/13	84.6	1/13	7.7	1/13	7.7
Plovdiv/Bolyantsi	14/17	82.4	2/17	11.7	1/17	5.9
Total	25/30	83.3	3/30	10.0	2/30	6.7
Pazardzhik/Byaga	10/12	83.3	2/12	16.7	0/12	0.0
Pazardzhik/Dragor	8/8	100.0	0/8	0.0	0/8	0.0
Total	18/20	90.0	2/20	10.0	0/20	0.0
Stara Zagora/Cherganovo	5/6	83.3	0/6	0.0	1/6	16.7
Stara Zagora/Vetren	12/14	85.7	1/14	7.1	1/14	7.1
Total	17/20	85.0	1/20	5.0	2/20	10.0
	60/70	85.7	6/70	8.57	4/70	5.7

N – number of the tested samples, n – number of the positive samples

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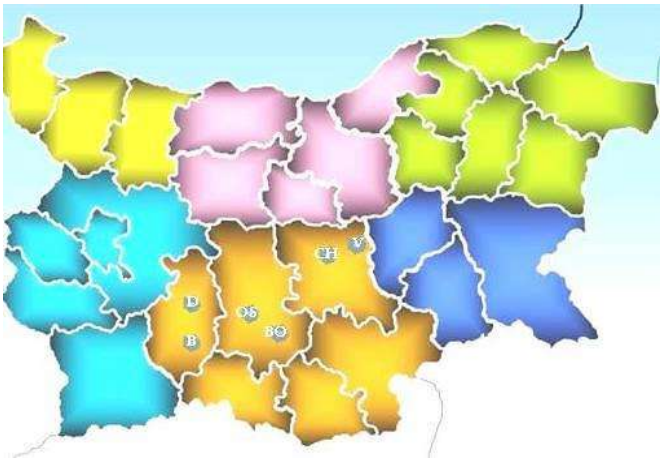

Fig. 1. Indicative map of the surveyed locations. B – Byaga, Pazardzik province; D – Dragor, Pazardzik province; BO – Boyantsi, Plovdiv province; OS – Ostromila, Plovdiv province; CH – Cherganovo, Stara Zagora province; V – Vetren, Stara Zagora province

Fig. 2. Symptoms on the fruits of PPV infected peach and nectarine. A – 'Glohaven' cv. fruit infected only by PPV-M; B – 'Adriana' cv. fruit co-infected by PPV-M and PPV-D; C – Elite 17-98 fruit infected only by PPV-D