

Low incidence of *Peach latent mosaic viroid* in peach mother blocks in Serbia

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SUMMARY

Peach latent mosaic viroid (PLMVd) is the causal agent of peach latent mosaic disease that is common on peaches and nectarines worldwide. Most of the isolates do not cause any symptoms on the foliage and the disease may be latent for years. A survey to investigate the presence of PLMVd in selected peach mother blocks in 9 Serbian districts was carried out in 2011 through 2013. A total of 315 trees/samples originating from 43 mother blocks, representing 35 peach and nectarine varieties and 2 rootstocks, were tested by Reverse Transcription - Polymerase Chain Reaction (RT-PCR). PLMVd was detected in 13 samples (4.13%) belonging to 7 varieties and one vineyard peach rootstock. Infected samples were found in 7 mother blocks from 3 districts. Our results indicated a low incidence of PLMVd in the analyzed peach mother blocks.

Keywords: *Peach latent mosaic viroid*; Peach mother blocks; Serbia

INTRODUCTION

Viroids are small, usually 246–401 nucleotides (nt), single-stranded, circular RNA molecules that infect plants and may cause significant losses in sensitive species and varieties. Viroids do not encode any pathogen-specific peptides; they replicate autonomously and spread within the plant by recruiting host proteins via functional motifs encoded in their RNA genome. Viroids are divided in two families: *Avsunviroidae* and *Pospiviroidae* (Flores et al., 2011). Members of the *Avsunviroidae* family are able to catalyze self-cleavage of multimers produced during replication and do not possess a central conserved region (CCR). Members of the *Pospiviroidae* family possess a CCR and have no self-cleaving properties.

Peach latent mosaic disease, caused by *Peach latent mosaic viroid* (PLMVd), was first reported in France more than 30 years ago on propagation material imported from the USA and Japan (Desvignes, 1976). However, related diseases had been reported in the United States much earlier as peach calico (1944) and peach blotch (1946), and as peach yellow mosaic (1973) in Japan (Flores et al., 2011). PLMVd, usually a 335–339-long nucleotide (nt), is a typical species of the genus *Pelamoviroid* (family *Avsunviroidae*). Cloning and sequencing of PLMVd variants of asymptomatic and mosaic-inducing peach isolates have revealed a wide range of variants with sizes between 335 and 351 nt (Pelchat et al., 2000; Malfitano et al., 2003). PLMVd does not have a central conserved region (CCR) and has acquired self-cleaving properties by forming hammerhead ribozyme structures (Flores & Llácer, 1989).

This disease is generally latent for 5-7 years before symptoms appear (Flores & Llácer, 1989). Most PLMVd isolates do not induce foliar symptoms. In sensitive varieties, some isolates may cause mosaic, blotch, vein banding or calico symptoms on infected leaves (Figures 1 and 2). Buds may show necrosis and shoot development is delayed. In the most serious cases branches may become necrotic and die off. Fruit symptoms include circular discolored areas, deformation of fruits with crack sutures and enlarged pits. PLMVd naturally infects stone fruits: apricot (*Prunus armeniaca*), peach (*P. persica*), sweet cherry (*P. avium*), plum (*P. domestica*) and almond (*P. amygdalus*), as well as pome fruits: cultivated pear (*Pyrus communis*) and wild pear (*P. amygdaliformis*) (Giunchedi et al., 2011). Peach latent mosaic disease occurs worldwide. The incidence of PLMVd in germplasm of different geographical origins is generally high and ranges from 40-90% (Fekih Hassen et al., 2007).



Figure 1. Blotching on vineyard peach leaves induced by *Peach latent mosaic viroid* isolate SD1



Figure 2. Mosaic symptoms on vineyard peach leaves caused by *Peach latent mosaic viroid* isolate SD1

Numerous studies have shown that PLMVd is transmitted by grafting, budding, contaminated blades or pollen, but not by seed (Hadidi et al., 1997; Desvignes et al., 1999; Barba et al., 2007; Faggioli & Barba, 2008). PLMVd has been experimentally

transmitted to healthy plants in the glasshouse using the aphid *Myzus persicae* but in general little is known of its epidemiology.

PLMVd was detected for the first time in Serbia in two peach varieties in the early 2000s (Jevremović & Paunović, 2006). In later studies, PLMVd was detected in more than half of the analyzed peach varieties from stone fruit collections (Mandić et al., 2008). PLMVd is a pathogen covered by special supervision of stone fruit mother blocks as regulated by the Ministry of Agriculture and Environmental Protection of the Republic of Serbia.

In this paper, we present the results of a three-year first survey for PLMVd in peach mother blocks.

MATERIAL AND METHODS

Peach sampling

A first survey for PLMVd in peach mother blocks in Serbia was carried out from 2011 through 2013. The survey was performed on 43 mother blocks from 9 districts: Belgrade, Bor, Central Banat, Kolubara, Mačva, Podunavlje, Rasina, South Bačka and Šumadija. Young fresh leaves were collected from 315 trees each year in June. Thirty-five peach and nectarine varieties (*Prunus persica* (L.) Batsch) and two rootstocks (GF677 - *Prunus persica* × *Prunus amygdalus* and vineyard peach) were included in this study (Table 1). Depending on the number of trees and varieties per block, 1-32 trees were randomly selected per block. Relative prevalence of PLMVd (Table 2) in the surveyed districts was shown as the number of infected mother blocks divided by the total number of tested mother blocks per district. PLMVd incidence (Table 2) was calculated as the number of infected trees divided by the total number of tested trees per district. The infection rate of varieties and samples (Table 3) was presented as a percentage of PLMVd positive varieties/samples.

Tissues of healthy peach seedlings, grown in glasshouses, were used as negative control. The PLMVd isolate SD1 (GenBank accession number JF416648), maintained on peach in the glasshouse, was used as a positive control (Jevremović & Paunović, 2008).

Total nucleic acid extraction

Total nucleic acids (TNA) were extracted from fresh peach leaves using a modified CTAB method described by Li et al. (2008). Leaf tissue (100 mg)

was ground in 1 ml of CTAB buffer (2% CTAB, 2% PVP-40, 100mM Tris–HCl, pH 8.0, 1.4M NaCl, 20mM EDTA, and 0.2% β -mercaptoethanol). The homogenate was incubated at 65°C for 15 min and centrifuged at 10,000 g for 5 min. An amount of 650 μ l of supernatant was transferred to each new 1.5 ml Eppendorf tube and mixed with the equal volume of 24/1 mixture of chloroform/isoamyl alcohol. The mixture was centrifuged at 15,000 g for 10 min. The obtained supernatant (\approx 500 μ l) was transferred to each new 1.5 ml Eppendorf tube with 350 μ l of isopropanol, mixed by pipetting and centrifuged at 15,000 g for 10 min. The pellet was rinsed with 1 ml of ice-cold 70% ethanol, centrifuged at 15,000 g for 5 min and dried at room temperature. Dried pellet was resolved in 100 μ l of 20mM Tris–HCl, pH 8.0.

RT-PCR amplification

TNA extracts were diluted (1:10) and amplified in Reverse Transcription and Polymerase Chain Reaction (RT-PCR) with One Step RT-PCR Kit (QIAGEN, Germany). To obtain a full-length amplified PCR product, PLMVd-c (5' -AAC TGC AGT GCT CCG T- 3') and PLMVd-h (5' -CCC GAT AGA AAG GCT AAG CAC CTC G- 3') primers were used (Shamloul et al., 1995). The RT reaction was performed at 50°C for 30 min, followed by initial PCR activation step at 95°C for 15 min. The PCR cycling parameters were as follows: 30 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 45 s, and a final extension at 72°C for 7 minutes.

PCR amplified products were analyzed by electrophoresis on 1.5% agarose gel in 0.5 x Tris/Borate/EDTA buffer (TBE) at 100 V for 30 min and by ethidium bromide staining.

Each RT-PCR run included a negative and a positive control.

RESULTS

PLMVd distribution in surveyed districts

The collected samples did not show any symptoms of mosaic, blotch, vein banding or calico that may indicate the presence of PLMVd. RT-PCR analysis revealed PLMVd presence in 13 out of 315 leaf samples (Table 1).

Table 1. The list of peach and nectarine varieties and rootstocks analyzed in the study

No.	Variety/rootstock	Number of analyzed samples	Number of PLMVd positive samples
1	Andross	1	–
2	Armgold	3	–
3	Baby Gold 6	1	–
4	Big Top	1	–
5	Caldesi	1	–
6	Caldesi 2000	3	–
7	Caldesi 2020	8	–
8	Cardinal	10	1
9	Collins	1	–
10	Cresthaven	11	1
11	Crimson Gold	1	–
12	Dixired	8	–
13	Early Redhaven	5	–
14	Elberta	1	–
15	Fantasia	35	6
16	Fayette	4	–
17	GF677	31	–
18	Glohaven	7	–
19	Independence	8	1
20	J. H. Hale	6	–
21	Maja	1	–
22	Maria Delizia	3	–
23	Nectared 4	10	–
24	Radmilovčanka	2	1
26	Red Top	5	–
25	Redcap	2	–
27	Redhaven	39	–
28	Southland	2	–
29	Springbelle	5	–
30	Springcrest	18	–
31	Springold	11	1
32	Springtime	7	–
33	Stark Red Gold	14	–
34	Suncrest	4	1
35	Super Queen	1	–
36	Vineyard peach	44	1
37	Vivian	1	–
	Total number	315	13

Table 2. Peach latent mosaic viroid (PLMVd) relative prevalence and incidence in surveyed districts

District	Number of mother blocks/samples	PLMVd relative prevalence (%)	PLMVd incidence (%)
Belgrade	1/13	–	–
Bor	1/1	–	–
Central Banat	3/21	33.33	19.05
Kolubara	1/5	–	–
Mačva	1/6	–	–
Podunavlje	1/5	–	–
Rasina	25/90	20.00	7.78
South Bačka	7/121	–	–
Šumadija	3/53	33.33	3.70
Total number	43/315		

Table 3. Peach latent mosaic viroid (PLMVd) infection rates of the analyzed varieties and samples in three districts

District	Peach mother block	Number of tested varieties/samples	Varieties infection rate (%)	Samples infection rate (%)
Central Banat	1	2/5	100.0	80
Rasina	2	7/8	14.3	12.5
	3	2/2	50.0	50.0
	4	4/4	25.0	25.0
	5	7/8	14.3	12.5
	6	6/11	50.0	27.3
Šumadija	7	10/26	20.0	7.7

The presence of PLMVd in peach mother blocks was confirmed in three districts: Central Banat, Rasina and Šumadija. Most of the affected plantations (5) were located in Rasina District, and another two in Central Banat and Šumadija Districts. PLMVd relative prevalence in the three districts ranged from 20-33.3% and its incidence from 3.77-19.05% (Table 2).

PLMVd infection rate in peach mother blocks

PLMVd was confirmed in 7 out of 43 tested mother blocks (16.27%). The PLMVd infection rate of the varieties of infected mother blocks ranged from 14.3% to 100% (Table 3). The lowest variety infection rate was detected in two mother blocks from Rasina District, and the highest in a mother block from Central Banat District. The infection rate of samples ranged from 7.7% to 80% in mother blocks from Šumadija and Central Banat Districts, respectively.

PLMVd incidence in peach varieties

The presence of PLMVd was analyzed in 35 different peach and nectarine varieties and two peach rootstocks. The analysis showed that PLMVd infection was

present in 7 varieties: Cardinal, Cresthaven, Fantasia, Independence, Radmilovčanka, Springgold and Suncrest (Table 1). PLMVd was also detected in one of the peach rootstocks. One out of 44 tested samples of vineyard peach was positive on PLMVd, but all 31 analyzed samples of the hybrid peach rootstock GF677 proved to be free of PLMVd. The incidence of PLMVd per infected variety and rootstock ranged from 2.3% to 50% in vineyard peach and Radmilovčanka, respectively.

DISCUSSION

Until 2005, the only category of fruit plant material produced in Serbia was standard 'CAC' material that does not derive from the 'basic' material. Strict control of the fruit plant material production has improved its quality (health status and trueness-to-type) in the last decade. A major problem in stone fruit production is *Plum pox virus* (PPV) that causes Sharka - the most detrimental viral disease of stone fruits. In the past, PPV had been occasionally found in stone fruits mother blocks in nurseries as well. Using strict sanitary control procedures the incidence of PPV and other economically important viruses in mother blocks has significantly decreased.

Peach latent mosaic viroid is a pathogen listed in the certification scheme for almond, apricot, peach and plum recommended by the European and Mediterranean Plant Protection Organization – EPPO (Anonymous, 2001), and in a Serbian bylaw on health inspection of crops and facilities for seed, replant and planting material production and health inspection of seeds, replants and planting material (Pravilnik, 2008). This 3-year survey was the first inspection of fruit mother blocks for the presence of viroids. The results were analyzed to show the relative prevalence and incidence of PLMVd in peach varieties and rootstocks in mother blocks in 9 Serbian districts. The results of the survey showed very low incidence (4.13%) of PLMVd in the analyzed samples. PLMVd was detected in 16.27% of the analyzed peach mother blocks. A significant percentage (20%) of the analyzed varieties was infected but not a single variety showed any leaf symptoms that could be ascribed to PLMVd infection.

The first finding of PLMVd on peach in Serbia had been reported by Jevremović and Paunović (2008) on two varieties in commercial orchards that showed clear symptoms on fruits. Afterwards, in a more comprehensive study, Mandić et al. (2008) reported high PLMVd incidence (50%) in collection orchards. The viroid incidence in collections may be attributed to an initial introduction of contaminated plants. The presence of PLMVd in commercial orchards in Serbia should probably be also attributed to contaminated material that had been grown for years in orchards allowing dissemination. The main route of PLMVd dispersion worldwide is through contaminated buds. Symptoms take more than 2 years to develop in the field, even after planting infected plant material (Flores et al., 2006). PLMVd infection is also transmitted locally by pruning tools and aphids, but less frequently (Faggioli & Barba, 2008). Even if a tree is infected, PLMVd is not transmitted from contaminated seeds to seedlings (Barba et al., 2007). Seedlings of vineyard peach are the most common generative propagated rootstock for peach in Serbia, but PLMVd incidence in vineyard peach was proved in our survey to be low (2.3%).

In conclusion, this study demonstrated a low incidence of *Peach latent mosaic viroid* in the tested Serbian peach mother blocks. The compliance with the latest legal regulations in practice has contributed to an increase in the quality of planting material. Analyses performed over the past years have reduced the incidence of several virus diseases in mother blocks. This survey of PLMVd presence in peach mother blocks contributed to an improved sanitary status of peach propagating materials in Serbia.

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Niska prisutnost viroida latentnog mozaika breskve u matičnim zasadima breskve u Srbiji

REZIME

Viroid latentnog mozaika breskve (*Peach latent mosaic viroid*-PLMVd) je prouzrokovatelj bolesti latentnog mozaika breskve koja se najčešće javlja kod breskve i nektarine širom sveta. Većina izolata ne izaziva nikakve simptome na lišću i bolest može biti latentno prisutna godinama. Tokom 2011-2013 godine sprovedena su istraživanja prisustva PLMVd u odabranim matičnim zasadima breskve u 9 okruga u Srbiji. Primenom metode reverzne transkripcije i lančane reakcije polimeraze (RT-PCR) analizirano je ukupno 315 stabala/uzoraka 35 sorti breskve i nektarine i 2 uzorka podloge iz 43 matičnih zasada. Prisustvo PLMVd je potvrđeno kod 13 uzoraka (4,13%) poreklom iz 7 sorti i jedne podloge vinogradske breskve. Zaraženi uzorci su utvrđeni u 7 matičnih zasada breskve iz 3 okruga. Dobijeni rezultati su pokazali nizak procenat prisustva PLMVd u analiziranim matičnim zasadima breskve.

Cljučne reči: Viroid latentnog mozaika breskve; matični zasadi breskve; Srbija