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PLUM POX VIRUS PRESENCE IN AUTOCHTHONOUS STONE FRUIT COLLECTION

Biljana Lolić*, Predrag Ilić, Stefani Tepić

University of Banja Luka, Institute of genetic resources, University City, Bulevar vojvode Petra Bojovića 1A, Republic of Srpska
*corresponding author:biljana.lolic@igr.unibl.org

ABSTRACT

Plum pox virus (PPV), also called Sharka belongs to Potyvirus genus, is the most devastating viral disease of stone fruit worldwide. Long distance spread is the result of moving infected nursery stock or propagative material, so grafts and budwood are also ways of moving infected material. Short distance spread is through aphid carriers. During 2024, a survey was performed to detect the presence of *Plum pox virus* (PPV) in stone fruits collection orchards in Aleksandrovac and at the site of Botanical Garden of the University of Banjaluka. In June, leaves plant tissues were collected and analyzed serologically by DAS-ELISA method with commercial antisera according to recommended protocol (Bioreba, Switzerland). A total of 38 accessions were analyzed where every sample include at least 2 trees. Plum, cherry, sour cherry, apricot and peach samples were collected, but all positive samples were from different accessions of plum trees. In total, 16 (42%) of symptom and symptomless plum accessions resulted as positive. The aim of this study was to observe the presence of Plum pox virus for further propagation and recovery procedures of autochthonous accessions of different stone fruits that exist in the collection of the Institute of Genetic Resources.

Key words: PPV, DAS-ELISA, *Prunus*, aphids.

INTRODUCTION

The main horticultural production in the Republic of Srpska is supplied by fruit-trees, particularly stone and pome fruits. The stone fruit industry, especially plum, has a long tradition in the use of different products. In 2020, plum production as the leading stone fruit crop was 51.426 t (Anonymous, 2020). Although, plum production has declined gradually in recent times and the main was due to "Sharka", which forced eradication of a huge number of trees of the most widespread local, but susceptible cultivar "Pozegaca". It also infects wild and ornamental *Prunus* trees and has a large experimental host range in herbaceous species.
Sharka disease was first reported in Bulgaria (at beginning of the 20th century), from where it spread progressively to most European countries (Roy and Smith, 1994). In nature, PPV is confined to the genus *Prunus*: plum, peach, apricot and most *Prunus* rootstocks. The disease is very detrimental in apricot, peach and plum trees because it produces reduced quality and premature dropping of fruits. Natural infections by PPV were found in sour cherry (Kalashyan and Bilkey, 1989; Nemchinov and Hadidi, 1996) and sweet cherry (Crescenzi et al., 1995). Almond is susceptible without showing symptoms (Németh, 1986).

The long distance spread of PPV is through infected nursery stock (rootstock, grafts, budwood) and propagating material. Short distance spread is by several aphid vectors by which PPV is transmitted in a non-persistent manner (Labonne et al., 1995). Vector species include *Myzus persicae* (Kassanis and Sutic, 1965), *Aphis spiraecola* (Leclant, 1973). In areas with a certain level of incidence of PPV infection, aphid transmission may increase the number of

infected trees in a few years. There is no confirmed evidence for seed or pollen transmission of PPV in any of its *Prunus* hosts (Pasquini and Barba, 2006).

Symptoms vary according to species and cultivar, virus strain, locality and seasonal conditions (Dosba et al., 1986; Kegler and Hartmann, 1998). Leaf symptoms consist of chlorosis (occasionally with necrotic spots), band and ring patterns or yellowing of secondary and tertiary veins. Plum and apricot fruits are deformed with internal flesh browning. Apricot stones are often marked by pale yellow rings (Dunez, 1987).

In the previous period, determination of the presence and distribution of stone fruit viruses was done in territory the Republic of Srpska as a part of Bosnia and Herzegovina (Matic, 2008, Matic et al., 2004) and location of Botanical Garden (Đuric et al., 2015). The identification of natural resistance in *Prunus* germplasm and its introduction into commercial cultivars by conventional breeding is one of the main strategies to control PPV, especially in areas of endemicity (Decroocq et al., 2011).

PPV has a consequence of both its high socioeconomic impact in the affected *Prunus* crops and its quarantine regulatory status in many countries as well as the Republic of Srpska.

METERIALS AND METHODS

Plant material

PPV causes sharka, the most damaging viral disease of stone fruit trees. The research was conducted on 38 accessions of autochthonous stone fruits from the Gene Bank of the Republic of Srpska. Collection orchards located in two sites Aleksandrovac and Botanical Garden of the University of Banjaluka were produced according to standard agricultural technology. All plants trees were maintained using standard horticultural practices such as pruning, fertilization and pest protection.

Leaf symptoms on infected trees included light chlorotic or yellow rings, spots and blotches, yellow line patterns along veins, vein clearing and leaf distortion. All plants were tested for the presence of PPV, no matter if leaf symptoms were present.

Sample preparation

In the end of June in 2024, monitoring and sample collection were carried out in 22 sites: stone fruits collection orchards in Aleksandrovac and in Botanical Garden of Institute of genetic resources, University of Banjaluka.

Figure 1. Leaf symptoms on Madjarica

Figure 2. Leaf symptoms on Prskulja

Fresh leaf samples were homogenized using Bioreba extraction bags. One sample for DAS-ELISA testing includes at least two stone fruit trees per accession of the same variety. Prepared samples were stored refrigerator at temperature 4°C over night.

Serological analysis

ELISA is highly specific and is recommended when low prevalence of PPV is expected (Garcia et al., 2014); moreover, it is sufficiently sensitive to consistently detect PPV during period of spring and summer. All collected samples were tested for the presence of *Plum pox virus* PPV by double antibody sandwich ELISA (DAS-ELISA) (Clark and Adams, 1977) with the reagents of BIOREBA AG (Switzerland) according to the manufacturer's recommendation. Serological reactants were provided by commercially purchased kits (Bioreba, Switzerland). Leaf tissue was extracted in extraction buffer (PBS-Tween + 2% PVP) in 1/20 (v/w) ratio. For DAS-ELISA, the wells of polystyrene plates were coated with IgG diluted in coating buffer and incubated at 37°C for 2h. The plates were washed three times for 3 min with washing buffer. After, the samples were loaded (200 µl per well), the plates were incubated for 2h at 37°C or overnight at 4°C. Following a wash, 200 µl of conjugated antibodies were added per well and the plates were incubated for 2h at 37°C. After three washes, 200 µl of freshly prepared p-nitrophenylphosphate in substrate buffer (1mg/ml) was placed in each well. The plates were incubated at room temperature and photometrically measured at 405 nm with an ELISA reader (BioSan, HiPo MPP-96) after 1h and 2h. Samples were considered positive if the OD values were two or more times higher than the OD values of the negative control.

RESULTS and DISCUSSION

The results of distribution of PPV in autochthonous stone fruit collection are summarized in the Table l. Samples of symptomatic and asymptomatic autochthonous stone fruit trees were investigated for the presence PPV in the orchard and compared with the laboratory results. The analysis of collected samples was performed by DAS-ELISA test. ELISA test, that was first applied in plant virology for the detection of PPV, is now used in routine testing to confirm the presence/absence of the virus even at low concentrations in different plant organs (roots, bark, flowers, leaves, fruits or seeds) (Adams, 1978). In this research, only the leaf samples were used for the laboratory approach. The leaves were collected in different part of the tree trying to find and collect symptomatic one. The most appropriate samples during vegetative period are leaves, especially in the period of May–June.

Table 1. Virus infection detected by DAS-ELISA in autochthonous stone fruit collection

Location: Aleksandrovac				
Cultivar		Symptoms	Positive	Negative
1.	Cherry Rani rust	no	0	4
2.	Cherry Crni rust	no	0	2
3.	Cherry Kasni rust	no	0	3
4.	Cherry Divlja crna	no	0	2
5.	Cherry Bijela aslama	no	0	3
6.	Sour cherry Buzim	no	0	3
7.	Peach - no name	no	0	2
8.	Apricot – no name	no	0	3
9.	Plum Nebozica	no	0	4
10.	Plum Bjelosljiva	no	0	2
11.	Plum Kavrkinja	yes	1	1
12.	Plum Kujina sisa	no	0	3
13.	Plum Bjelica rana	yes	1	2
14.	Plum Bijela sljiva	no	0	3
15.	Plum Cavka plava	no	0	1

16.	Plum Drizgulja	no	0	2
17.	Plum Azenka	no	3	0
18.	Plum Kajsijevaca	yes	1	0
19.	Plum Miskovacka rana	no	0	2
20.	Plum Madjarica	yes	1	1
Location: Botanical Garden of the University of Banjaluka				
Cultivar		Symptoms	Positive	Negative
21.	Apricot – no name	no	0	2
22.	Cherry Divlja crnica	no	0	2
23.	Cherry Bijelica	no	0	3
24.	Cherry Trijesanj bijeli	no	0	2
25.	Plum Divlja crnica	no	0	2
26.	Plum Bjelica rana	no	0	2
27.	Plum Turgulja	no	1	0
28.	Plum Dizgulja	yes	1	0
30.	Plum Nebozica	yes	1	1
31.	Plum Bjelosljiva	yes	1	1
32.	Plum Pozegaca bijela	no	1	0
33.	Plum Banjalucka bjelica	yes	1	3
34.	Plum Bijela pozegaca	no	0	2
35.	Plum Prskulja	yes	1	2
36.	Plum Drizgulja	no	0	1
37.	Plum Kujina sisa	yes	1	0
38.	Plum Rana	no	1	0

The presence of PPV is found in 14 out of 38 (42%) from autochthonous plum genotypes tested by DAS-ELISA. Only plum accessions resulted as positive. OD values of the PPV positive samples were 2–10 times higher than the OD values of the negative control.
Accessions variety: Azenka, Turgulja, Pozegaca bijela and Rana were symptomless but positive by DAS-ELISA test. Other plum accession variety: Kavrkinja, Bjelica rana, Kajsijevaca, Madjarica, Dizgulja, Nebozica, Bjelosljiva, Banjalucka bjelica, Prskulja and Kujina sisa show the symptoms like mild light green discoloration bordering the leaf veins (vein yellowing) and yellow to light green rings and positive by DAS-ELISA test. All 3 plum tress of Azenka variety were positive for the presence of PPV.

In the absence of resistant cultivars in domestic plum, tolerant cultivars that do not display fruit symptoms, but also do not restrict PPV multiplication and movement. To avoid PPV spread over long distances by the movement of plant material, reliable detection methods are needed for the accurate detection of the virus in symptomless nursery plants and propagative material. The recommended method by EPPO include also serological assays as well as sampling, reagents and detailed protocols.

The diversity of PPV isolates and its wide presence in Bosnia and Herzegovina was reported earlier by Matic et al. (2005). The wide presence of sharka was also reported in neighboring countries: Albania (Myrta et al., 1994), Bulgaria (Kamenova et al., 2003), Croatia (Kajic et al., 2008), Montenegro (Virscek-Marn et al., 2012) and Serbia (Jevremovic, 2008).

CONCLUSION

These results showed that some genotypes of autochthonous plum cultivars can be found free of PPV, even grown in the vicinity of PPV infected trees. This is of the great importance in order to preserve these PPV-free genotypes in proper isolated gene bank collections. The revitalization of the stone fruit sector will require a significant effort, whereby support for local

nurseries to produce the needed quantities of healthy planting stocks is of paramount importance. Virus control in countries where the disease is not present are based on quarantine measures to avoid its introduction. In countries where the level of infection is low and infected trees are restricted to limited areas, eradication has made it possible to maintain a low level of infection and more rarely to eliminate the disease. Under these conditions, the only possibility to cultivate stone fruit trees will be the use of more tolerant cultivars. Conventional breeding for sharka resistance has been hampered by the scarcity of resistant cultivars and by the polygenic nature of the resistance traits in the cases that have been studied (Dosba et al., 1992, Badenes et al., 1996).

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