First report of Plum bark necrosis stem pitting-associated virus in Bulgaria detected by DAS-ELISA

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Abstract

Plum bark necrosis stem pitting-associated virus (PBNSPaV), the causal agent of plum bark necrosis stem pitting disease, belongs to the genus *Ampelovirus* in the family *Closteroviridae*. In the past 20 years, the virus was reported in Europe, North Africa, the Middle East, and Asia. This study focused on occurrence and distribution of PBNSPaV in collection and commercial stone fruit orchards in Kyustendil region of Bulgaria. In two consecutive years 2017–2018, a total of 127 trees from 8 different stone fruit species were tested by DAS-ELISA for the presence of PBNSPaV. The virus was detected in eight symptomatic trees with bark gummosis, necrosis, pits and grooves on the branches and trunk of newly introduced Japanese plum (*Prunus salicina* Lindl.) cultivars. The established 6.3 % rate of infection showed a weak infestation with the virus. To the best of our knowledge, this is the first report of PBNSPaV in Bulgaria.

Key words: ELISA; stone fruit species; PBNSPaV; symptoms; virus detection

INTRODUCTION

Plum bark necrosis stem pitting (PBNSP) disease was first observed in 1986 on a 'Black Beaut' Japanese plum (*Prunus salicina* Lindl.) in the United States (Uyemoto & Teviotdale, 1996). This was the first confirmation of graft-transmissibility of the disease. A disorder resembling the *P. salicina* disease was observed in 1995 in Apulia (Southern Italy) in apricot of cv. Tyrinthos grafted on myrobolan (Di Terlizzi & Savino, 1995). Later in 2002, an associated virus, and the presumed causal agent, was partially characterized and named *Plum bark necrosis stem pitting-associated virus* (PBNSPaV) (Marini et al., 2002). PBNSPaV is a member of the genus *Ampelovirus*, family *Closteroviridae* (Martelli et al., 2005) with filamentous particles *ca*. 1500 nm in length (Amenduni et al., 2005).

To date, the disease was reported in Italy (Ghanem-Sabanadzovic et al., 2001), Morocco (Bouani et al., 2004), Serbia (Mandic et al., 2005), Jordan (Sánchez-Navarro et al., 2005), Egypt (Maghraby et al., 2007), Turkey (Usta et al., 2007), France (Marais et al., 2009), Spain (Garcia-Ibarra et al., 2010), Tunisia (Salleh et al., 2011), China (Cui et al., 2011), Korea (Jo et al., 2016) and Japan (Candresse et al., 2017). PBNSPaV was identified in many cultivated and ornamental *Prunus* species, including Japanese and European plums (*P. salicina* and *P. domestica*), apricot (*P. armeniaca*), peach (*P. persica*), sweet and sour cherry (*P. Avium* and *P. cerasus*), and almond (*P. dulcis*) (Amenduni et al., 2005; Marini et al., 2002; Abou Ghanem-Sabanadzovic et al., 2001; Usta et al., 2007; Mandic et al., 2007). The virus causes bark necrosis and gummosis on scaffold branches and the main trunk, and stem pitting. The symptoms of PBNSPaV greatly depend on the susceptibility of the hosts and the cultivars (Boscia et al., 2011).

The presented investigation was focused on occurrence and distribution of PBNSPaV in collection and commercial stone fruit orchards in Kyustendil region of Bulgaria.

MATERIALS AND METHODS

Plant material

In two consecutive years 2017–2018, one hundred and twenty-seven sweet cherry, ornamental cherry and sour cherry, European and Japanese plum, peach, apricot and *Pr. cerasifera* trees were visually inspected and sampled. The samples were collected from the stone fruit trees growing in four collection orchards located at the Institute of Agriculture – Kyustendil and two commercial orchards inthe region. The plum collection orchards were established in 1994 and 2011, and sweet cherry orchards in 2002 and 2012, respectively. The samples belonged to different species and cultivars of different origin, from the international exchange activity of the Institute of Agriculture – Kyustendil and also from the native cultivars (Table 1.). Most of the samples were collected from trees showing decline, gummosis, flattening of scaffold branches, necrosis of bark tissues, and some samples were gathered from trees showing no virus-like symptoms.

Serological assays

Phloem and/or leaf tissue were collected randomly from different branches around and across the tree in late autumn (late October and November) or spring (April). All samples were tested by DAS-ELISA (Clark & Adams, 1977) using specific antibodies against PBNSPaV (Agritest, Italy) according to the manufacturer's instructions. Both specific and conjugated antibodies were used in quantity of 100 µl, incubated at 37ºC for 2h. 150 µl of diluted 1:20 extracts (w/vol) and were loaded in duplicate wells of polystyrene microtiter plate for overnight incubation at 4ºC. Between each step, the plate was washed 3 times with PBS-T (phosphate-buffered saline-Tween). 100 µl freshly prepared p-nitrophenylphosphate in substrate buffer (1mg/ml) was loaded to each well. The plate was incubated at room temperature and photometric measurement was done at 405 nm after 1-2 h. Samples were considered as positive, if their absorbance values were more than three times higher than the negative control.

RESULTS AND DISCUSSION

Field observations

During visual inspection, our attention was focused on trees with typical PBNSPaV symptoms as gummosis and bark necrosis of the trunk and the scaffold branches, as well as stem pitting (Di Terlizzi & Savino, 1995; Boscia et al., 2011). This kind of symptoms were seen in trees of *Prunus salicina* cultivars 'Qiuji', 'Zaoli' and 'Taoli' and consisted of bark gummosis, necrosis, pits and grooves on the branches (Fig.1; 2). The bark of the trunk and main branches showed extensive splitting (Fig. 3).

According to the obtained data from the serological analysis the cultivars 'Qiuji', 'Zaoli' and 'Taoli' were 100% infected with PBNSPaV, which helped us conclude that these symptoms were associated with PBNSPaV infection. In cultivars 'Qiuji' and 'Zaoli' were observed symptoms on leaves. They consisted of early bud break, followed by leaf reddening and rolling in the summer, but it should be noted, that European Stone Fruit Yellows (ESFY) phytoplasma was detected in these trees in our previous work (Borisova & Kamenova, 2016). These symptoms are typical for ESFY and do not correspond to PBNSPaV infection.

In some of the sweet cherry cultivars was observed necrosis on scaffold branches and trunks, sunken in appearance and associated with dark-colored gummosis, in some case leading to death of branches. Although symptoms on trunks and branches of trees of this species were identified, ELISA tests did not confirm the presence of PBNSPaV. These symptoms could due do to some other pathogens as *Pseudomonas syringasome e pv . syringae.*

Latent infections have been reported in some hosts or in specific cultivars of otherwise susceptible hosts (Boscia et al., 2011; Garcia-Ibarra et al., 2010). For that reason samples for serological analysis taken from cultivars of different species showing no symptoms. In our investigation, infection of PBNSPaV was not serologically proved in none of these symptomless samples.

Serological evaluation

The results of ELISA tests are shown in Table 1. All samples from fresh phloem tissue collected in late autumn from the Japanese plum, cv. 'Qiuji', 'Zaoli' and 'Taoli' tested positive for PBNSPaV. The results from ELISA tests of young leaves collected in the springfrom the same trees were also positive, but with lower absorbance values. According to Amenduni et. al. (2005) with some other closteroviruses, and particularly those infecting grapevines (Martelli et al., 1997), PBNSPaV particles are pres-

Table 1. Incidence of PBNSPaV in tested stone fruit species.

Species	Cultivar	Number tested	Number positive for PBNSPaV
Pr. avium (Sweet cherry)	Bing	10	0
	Kozerska	1	0
	Bigalise pozna	3	0
	Pobeda Krimska	1	0
	Vega	1	0
	Victoria	1	0
	Germersdorfer	5	0
	Tieton	3	0
	Star z chech	1	0
	Santina	1	0
	Superstar	1	0
	Van	4	0
	Merchant	1	0
	Drogan's Yellow	4	0
	Sparkle	2	0
	N8 -102	3	0
	Sam	2	0
	Summit	4	0
Pr. cerasus (Sour cherry)	Erdi Bötermö	2	0
Prunus serrulata (Ornamental cherry)	Kwanzan	7	0
Pr. domestica (European plum)	Top gigant plus	2	0
	Top hit	1	0
	Top	1	0
	Top 2000	1	0
	Toper	1	0
	Topking	2	0
	Elit 998	1	0
	Čačanska Najbolja	5	0
	Stanley	16	0
	Tuleu timpuriu	4	0
	unknown	4	0
Prunus salicina (Japanese or Chinese plum)	Qiuji	3	**3**
	Zaoli	3	**3**
	Taoli	2	**2**
	Mili	1	0
	Black Diamond	5	0
Prunus persica (Peach)	Glohaven	1	0
	Redhaven	2	0
	Hale	7	0
P. armeniaca (Apricot)	unknown	3	0
Prunus cerasifera	unknown	5	0
TOTAL		127	8 (**6,3 %**)

Figure 1. Necrosis, pits and grooves on the branches of tree 'Zaoli' Japanese plum, infected with PBNSPaV

Figure 2. Gummosis on a trunk of tree 'Zaoli' Japanese plum, infected with PBNSPaV

Figure 3. Extensive splitting on a trunk of tree *Pr. salicina* cultivar 'Qiuji', infected with PBNSPaV

ent in infected tissues at low concentrations in the early phases of growth but its titre increases progressively during the growing season, reaching a maximum at the end of summer. To our knowledge, this is the first report of PBNSPaV in Bulgaria.

No PBNSPaV infection was found in the sample from the other assayed trees of Japanese plum, in sweet, sour and ornamental cherries, European plum, apricot, peach and *Pr. cerasifera* (Table 1).

The incidence of PBNSPaV was 6,8%. The low incidence of virus together with its distribution in only eight Japanese plum trees could be due to the introduction of virus-contaminated scion buds. The virus is not mechanically transmitted and has no known vector, although natural virus spread was reported (Terlizzi & Savino, 1995). Its transfer from plant to plant is thought to occur through vegetative propagation techniques. As with other *Ampelovirus* spp., PBNSPaV is not known to be seed transmitted.

The relatively low rate of PBNSPaV presence in stone fruit species in Bulgaria is similar to the reported 2.5% infection in apricot in Spain (Garcia-Ibarra et al., 2010). Serological analysis showed that nearly 8% of the samples were infected by PBNSPaV, particularly

those from peach (15.7%), plum (11.1%) and almond (5.4%) trees in the study carried out in Tunisia (Salleh et al., 2011). PBNSPaV was found in different stone fruit samples with fairly high incidence of 26% in Italy, 40% in Egypt, 51% in Serbia and 70% in Turkey (Matic et al., 2010). High infection level detected in Turkey (77%) was reported also by Usta et al. (2007).

More sensitive methods as RT-PCR for further PBNSPaV detection in *Prunus* species in the country are desirable.

CONCLUSIONS

This is the first report of *Plum bark necrosis stem pitting-associated virus* (PBNSPaV) in Bulgaria identified by DAS-ELISA. The virus was detected in a few trees of newly introduced Japanese plum cultivars. The established 6.3 % rate of infection showed a weak infestation of the virus. Futher efforts are needed to understand distribution of PBNSPaV in Bulgaria and its contribution to syndroms affecting stone fruit trees.

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