Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2401111B
Vol. 69, No. 1, 2024 UDC: 634.232:[632.38:578.85/.86(497.2)
Pages 111-119 Preliminary communication

AN UPDATE ON APPLE CHLOROTIC LEAF SPOT VIRUS STATUS OF SWEET CHERRY IN BULGARIA

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Abstract: The sweet cherry (*Prunus avium* L.) is one of the most important stone fruit species in Bulgaria. The cherry is susceptible to many viruses. To gain a better insight into the phyto-virologic status of sweet cherry, a survey for the presence of apple chlorotic leaf spot virus (ACLSV), cherry leafroll virus (CLRVR) and raspberry ringspot virus (RpRSV), in addition to ilarviruses, was carried out in 32 commercial and 6 collection orchards in ten regions of Bulgaria between 2017 and 2022. A total of 1503 sweet cherry samples were collected from symptomatic and symptomless trees. These samples were tested by cocktail ELISA for ACLSV and DAS-ELISA for CLRVR, RpRSV and additionally for prune dwarf virus (PDV) and prunus necrotic ringspot virus (PNRSV) to detect mixed infections. Of the three viruses studied, only ACLSV was detected in commercial and collection sweet cherry orchards in all regions with intensive cherry cultivation in Bulgaria, in addition to ilarviruses. The extent of ACLSV virus infection was 8.8%. Single ACLSV infection was the most common, affecting 79.6% of infected sweet cherry trees, followed by ACLSV and PDV (14.4%) and least frequently by ACLSV and PNRSV (6.0%). The presence of ACLSV in orchards of different ages, including 'young' orchards, indicates that virus-free propagation material must be used when establishing new orchards to prevent the spread of pathogens with the planting material.

Key words: sweet cherry, ELISA, detection, viruses, ACLSV.

Introduction

The sweet cherry (*Prunus avium* L.) is one of the main fruit crops in Bulgaria in terms of cultivated area (12 649 ha in 2021) and production (52 615 t in 2021) (MA, 2022). The sweet cherry is mainly cultivated in southern Bulgaria.

The cherry is susceptible to many virus-associated diseases. More than 30 viruses have been reported, most of which belong to the *Ilarvirus, Potyvirus,* and *Trichovirus* genera (Myrta and Savino, 2008).

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Apple chlorotic leaf spot virus (ACLSV) of the genus *Trichovirus*, together with similar viruses – prune dwarf virus (PDV) and prunus necrotic ringspot virus (PNRSV), is one of the most common viruses in cherries (Mandic et al., 2007; Sánchez et al., 2015; Deltedesco et al., 2022). Generally, ACLSV-infected trees are symptomless, but incompatibility of scion-rootstock combinations in nurseries, deformation and discoloration, and necrosis of fruit in susceptible sweet cherry cultivars have been reported (Desvignes and Boye, 1989; Rana et al., 2011; Liu et al., 2014; Sánchez et al., 2015).

Cherries can also be infected by viruses belonging to the genus *Nepovirus.* Cherry leafroll virus (CLRV) and raspberry ringspot virus (RpRSV) are some of these nematode-borne viruses (Martelli and Uyemoto, 2011).

CLRV alone causes a slow decline in sweet cherries, but in mixed infections with either PDV or PNRSV, symptoms become much more severe and tree decline is accelerated (Eastwell and Howell, 2010; Lutes and Pscheidt, 2018). The symptoms induced by RpRSV-ch on sweet cherries can also be very severe, even leading to the death of the tree (Wetzel and Krczal, 2007).

In recent years, extensive studies have been conducted on the distribution of ilarviruses on sweet and sour cherries in Bulgaria (Kamenova et al., 2020; Borisova et al., 2021), while data on the spread of other viruses on cherries are insufficient and only available for individual regions and cultivars.

To gain a better insight into the sanitary status of sweet cherries, outside the ilarviruses, a survey was carried out for the presence of ACLSV, CLRVR, and RpRSV in commercial and collection orchards in different regions of Bulgaria.

Material and Methods

Sampling procedure and visual evaluation of virus symptoms

The surveys were carried out from April and May 2017 to May and June 2022 in several provinces, mainly in southern Bulgaria. Field inspections were conducted and samples were collected from thirty-two commercial and six collection sweet cherry orchards (four at the Institute of Agriculture (IA), Kyustendil, one at the Agriculture school, Kyustendil, and one at the Research Institute of Mountain Stockbreeding and Agriculture (RIMSA), Troyan) (Table 1). Twelve individual home-growing sweet cherry trees from the Kyustendil region were also included. A total of 1503 sweet cherry samples were collected from symptomatic and symptomless trees. Each tree in the field and each sample in the laboratory were examined for symptoms. All samples (petals and leaves) were taken randomly from different branches at different heights around each sampled tree.

Serological tests – enzyme-linked immunosorbent assay (ELISA)

The samples were labeled and placed in isolated transparent bags, transported to the virology laboratory, and maintained at 4°C. All samples were tested for the presence of five viruses (ACLSV, CLRVR, RpRSV, PNRSV, and PDV) within a few days after sampling using ELISA. Two ELISA variants were performed – for the identification of CLRV and RpRSV, the double antibody sandwich variant (Clark and Adams, 1977) was applied and for the detection of ACLSV, the test was carried out according to a modified procedure named cocktail ELISA developed by Flegg and Clark (1979). At the same time, the samples were also analyzed by DAS-ELISA for the presence of PNRSV and PDV viruses to detect mixed infections. The commercial diagnostics sets manufactured by Loewe Phytodiagnostica GmbH were applied, using the protocol recommended by the producer. Samples showing an optical density (OD) three times the average of the negative controls were considered as positive.

Results and Discussion

Surveys and symptoms in the field

The orchards for field observation and sample collection were selected in some of the regions with the most intensive sweet cherry cultivation in Bulgaria, such as Kyustendil, Sliven, Plovdiv, Stara Zagora, and Burgas. To represent the different types of orchards, old (over 50 years old) and abandoned plantations (Kyustendil and Kazanlak), newly established ones (1 to 5 years old in Blagoevdrad, Burgas and Sliven), and middle-aged (10–15 years old in Plovdiv, Burgas, Pazardzhik, Sliven, and Kyustendil) were included.

The sweet cherry trees in each orchard were initially monitored by visual observation for the development of virus-like symptoms. Most of the trees in the surveyed orchards did not show any symptoms that could suggest virus infections and samples were collected randomly. However, there were some cases in which typical symptoms of stone fruit viruses, such as chlorotic diffuse rings or spots, interveinal chlorosis, deformations, and wrinkling on the leaves were sporadically observed in some sweet cherry orchards surveyed. Chlorotic and necrotic spots and lines on the leaves of trees of the “Bing” cultivar were noted in an orchard in Kazanlak. Chlorosis, necrotic spots, deformation, and wrinkling on the leaves of trees of the “Kozerska”, “Lambert”, “Van” cultivars were seen in orchards in the Aytos, Kyustendil, and Kazanlak regions. These symptoms were found in a serologically proven mix of ACLSV + PDV or ACLSV+ PNRSV infected trees (Figure 1 B, C). Most of the single-infected ACLSV trees were symptomless. These results confirm reports of natural infections of stone fruit trees by ACLSV as

a latent virus (Nemeth, 1986; Sutic et al., 1999). Only in one case, ACLSV-infected trees showed symptoms in the form of rings and spots without necrosis around them on individual leaves of trees of the cultivar “Bigarreau Burlat” in the orchard of Gabarevo/ Kazanlak (Figure 1 A).

A B C

Figure 1. Symptoms on the leaves of sweet cherry trees. A/rings and spots on the leaf of the tree cultivar “Bigarreau Burlat” infected with ACLSV; B/chlorotic and necrotic spots and lines on the leaf of the tree cultivar “Bing” infected with ACLSV + PDV; C/chlorosis, deformation and wrinkling on the leaf of the tree cultivar “Kozerska” infected with ACLSV + PNRSV.

Most of the visual monitoring was carried out in April and May of 2017–2022, which did not allow the observation of symptoms on ripe cherry fruit. In our previous work, necrosis was found on sweet cherry fruit on trees infected with ACLSV (Borisova and Christov, 2014).

Serological detection

A total of 1503 samples of sweet cherry trees from ten different districts in Bulgaria were serologically analyzed for the presence of ACLSV, CLRV, and RpRSV, except for the presence of ilarviruses. The results of the ELISA tests showed that the samples tested were infected only with ACLSV in single or in mixed infection with ilarviruses. The presence of CLRV and RpRSV was not detected in any of the samples tested (Table 1).

The ELISA analysis revealed that 132 of 1503 tested samples were infected. The total infection rate was 8.8%, that with ACLSV was 7.0% and that with ACLSV+PDV/or PNRSV was 1.8% (Table 1).

Table 1. Extent of single and mixed infections with ACLSV in sweet cherry in the surveyed regions of Bulgaria.

Region/District	Orchard location	Samples		Positive for:	
		Number tested	Number infected (%)	ACLSV	ACLSV + PNRSV/or PDV
Southeastern/ Sliven	5 commercial orchards	166	17 (10.2)	17	0
Southeastern/ Burgas	4 commercial orchards (town Aytos)	160	9 (5.6)	3	5 (with PNRSV) 1 (with PDV)
Southeastern/ Burgas	4 commercial orchards (town Pomorie)	96	2 (2.1)	2	0
South-central/ Plovdiv	4 commercial orchards	132	12 (9.1)	11	1 (with PDV)
South-central/ Pazardzhik	1 commercial orchard (town Septemvri)	46	8 (17.4)	8	0
South-central/ Stara Zagora	2 commercial orchards (town Maglish)	81	1(1.2)	1	0
South-central/ Stara Zagora	3 commercial orchards (town Kazanlak)	135	34 (25.2)	30	3 (with PNRSV) 1 (with PDV)
Western/ Kyustendil	4 collection orchards, IA-Kyustendil	340	14 (4.1)	13	1 (with PDV)
Western/ Kyustendil	1 collection orchard, Agriculture School	35	2 (5.7)	2	0
Western/ Kyustendil	4 commercial orchards, single trees	103	14 (13.6)	9	5 (with PDV)
Western/ Dupnica	1 commercial orchard (town Bobov dol)	48	0	0	0
Western/ Pernik	1 commercial orchard (town Radomir)	47	17 (36.2)	7	10 (with PDV)
Southwestern/ Blagoevgrad	3 commercial orchards	103	2 (1.9)	2	0
North-central/ Lovech	1 collection orchard, RIMSA-Troyan	11	0	0	0
Total		1503	132 (8.8)	105 (7.0)	27 (1.8)

The difference in ACLSV incidence among the orchards ranged from 1.2% to 36.2%. The highest infection rate of 36.2% (14.9% of single and 21.3% of mixed infection with PDV) was recorded in the “old” sweet cherry orchard located in Radomir, Pernik district, followed by the infection rate of 25.2% (22.2% of single infection, 2.2% of mixed infection with PNRSV, and 0.8% with PDV) found in the orchards in Kazanlak, Stara Zagora district. The lowest infection in sweet cherry trees (1.2% and 1.9%) was noted in “young” orchards in Maglish, Stara Zagora, and in orchards of different ages in Blagoevgrad, respectively. All tested sweet cherry trees from the collection orchards at RIMSA-Troyan and the commercial orchard in Bobov dol, Dupnica were free from the tested viruses. No differences in virus prevalence were found for the different age groups of the investigated orchards.

According to the preliminary data, 13.3% of the sweet cherry in the Kyustendil region of Bulgaria was infected with ACLSV, and 33.4% of the tested trees were found to be infected with ACLSV and PDV or PNRSV (Borisova, 2005). In sweet cherry orchards in Spain, the infection with this virus was 16.0% (Sánchez et al., 2015) and 14.0% in the Mediterranean region (Myrta and Savino, 2008). A lower level of infection (only 4.0%) with this pathogen was found in Serbia (Mandic et al., 2007). Similar results were obtained in the study of sweet cherry cultivars in Ukraine – 3.6% (Pavlikuk et al., 2021).

ACLSV was very often detected in mixed infections with ilarviruses, PDV or PNRSV, in sweet cherry (Ulubaş, 2008; Sánchez et al., 2015; Deltedesco et al., 2022). ACLSV and PDV were the viruses that occurred most frequently in combination, accounting for 14.4% of infected sweet cherry trees, followed by ACLSV and PNRSV (6.0%) in our study. A low infection rate for the PNRSV-ACLSV combination in cherry trees was also reported by Mandic et al. (2007) and Çevik et al. (2011) in Serbia and Turkey, respectively.

None of the tested samples were positive for CLRV and RpRSV and these results are in agreement with the reported absence of these viruses in stone fruit species in the Kyustendil region in Bulgaria (Borisova et al., 2013). However, in a study on sap-transmissible viruses in sweet cherries, Milusheva and Zivondov (2009) reported that CLRV was the most prevalent virus, identified in 31.9% of the trees tested, followed by RpRSV – 26.0% in the Plovdiv region. In another study, the most widespread virus was also CLRV, identified in single or mixed infection in 56.4% of the cherry samples tested in the same region (Milusheva et al., 2014). In our study, no trees infected with CLRV or RpRSV were found, although the Plovdiv region was included. Different rates of CLRV infection have been reported in different regions and countries. According to Tryapitsyna and Vasiuta (2010), 21.63% of sweet cherry orchards in Ukraine were infected with CLRV, while Pavlikuk et al. (2021) reported the presence of the pathogen in 0.7% of sweet cherries. In Romania, CLRSV was not detected in the investigated sweet cherry orchards (Zagrai et al., 2022).

Conclusion

In the present study, of the viruses tested in all regions with intensive cherry cultivation in Bulgaria, only ACLSV was detected in commercial and collection sweet cherry orchards in addition to ilarviruses. The extent of ACLSV virus infection in 1503 sweet cherry trees tested was 8.8%. Single infection with ACLSV was most common, accounting for 79.6% of infected sweet cherry trees, followed by ACLSV and PDV (14.4%) and least by ACLSV and PNRSV (6.0%). The presence of ACLSV in orchards of different ages, including “young” orchards, indicates that virus-free propagation material must be used when establishing new orchards to prevent the spread of pathogens with planting material.

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Received: May 12, 2023
Accepted: January 25, 2024

AŽURIRANJE STATUSA VIRUSA HLOROTIČNE LISNE PEGAVOSTI JABUKE NA TREŠNJI U BUGARSKOJ

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R e z i m e

Trešnja (*Prunus avium* L.) je jedna od najvažnijih vrsta koštičavog voća u Bugarskoj. Trešnja je domaćin brojnih virusa. U cilju dobijanja boljeg uvida u fito-virusni status trešnje, u periodu 2017.-2022. godina sprovedeno je istraživanje prisustva virusa hlorotične lisne pegavosti jabuke (engl. *apple chlorotic leaf spot virus* – ACLSV), virusa uvijanja lista trešnje (engl. *cherry leafroll virus* – CLRVR) i virusa prstenaste pegavosti maline (engl. *raspberry ringspot virus* – RpRSV), pored ilarvirusa, u 32 komercijalna voćnjaka i 6 kolekcionih zasada trešnje u deset regiona Bugarske. Prikupljeno je ukupno 1503 uzorka trešnje sa stabala sa i bez simptoma. Uzorci su testirani primenom koktel ELISA za ACLSV i DAS-ELISA metode za CLRVR, RpRSV, kao i za virus kržljavosti šljive (engl. *prune dwarf virus* – PDV) i virus nekrotične prstenaste pegavosti koštičavih voćaka (engl. *prunus necrotic ringspot virus* – PNRSV) u cilju detekcije mešanih infekcija. Pored ilarvirusa, od druga tri proučavana virusa, samo je ACLSV uočen u komercijalnim voćnjacima i kolekcionim ili matičnim zasadima trešnje u svim regionima sa intenzivnim uzgojem trešanja u Bugarskoj. Zastupljenost virusa hlorotične lisne pegavosti jabuke bila je 8,8%. Pojedinačna infekcija virusom hlorotične lisne pegavosti jabuke bila je najzastupljenija, detektovana u 79,6% zaraženih stabala trešnje, a zatim slede ACLSV i PDV (14,4%) i ACLSV i PNRSV (6,0%). Prisustvo virusa hlorotične lisne pegavosti jabuke u voćnjacima različite starosti, uključujući i „mlade" zasade, ukazuje na to da se mora koristiti bezvirusni sadni materijal prilikom zasnivanja novih voćnjaka, kako bi se sprečilo širenje patogena sadnim materijalom.

Ključne reči: trešnja, ELISA, detekcija, virusi, ACLSV.

Primljeno: 12. maja 2023.
Odobreno: 25. januara 2024.

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