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Incidence of ilarviruses in cherry nurseries and newly established orchards

Разпространение на ilarviruses в черешови разсадници и новосъздадени градини

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ABSTRACT

Sweet cherry (*Prunus avium* L.) is one of the most important stone fruit species in Bulgaria. The species is known to be susceptible to more than 30 viruses. Among them are the important prune dwarf virus (PDV), prunus necrotic ringspot virus (PNRSV) and apple mosaic virus (ApMV), ilarviruses. Testing for these viruses is required in the certification schemes in order to provide virus-tested propagated material to the fruit-growing industry and to prevent their distribution by imported infected plant materials. Visual field surveys, followed by DAS-ELISA tests of 664 leaf samples collected in 2019 and 2020 from cherry trees grown in several nurseries and young orchards were conducted. PDV and PNRSV were serologically detected. ApMV was not detected in any of the tested one-year-old seedlings and young trees. The overall infection rate was 11.4%. PDV was the most frequent (7.4%), both in nurseries (7.2%) and in 'young' orchards (7.6%), followed by PNRSV – 4.1% in nurseries and 4.0% in 'young' orchards. The results showed differences in the average occurrence among the viruses in nurseries and the orchards, ranging from 0.9 % in a nursery (Plovdiv district) to 31.6% in one-year-old orchard (Petrich region).

Keywords: Bulgaria, DAS-ELISA, infections, PNRSV, PDV, ApMV

РЕЗЮМЕ

Черешата (*Prunus avium* L.) е една от основните овощни култури в България. Тя се напада от над 30 вируса. Сред тях значими са prune dwarf virus (PDV), prunus necrotic ringspot virus (PNRSV) и apple mosaic virus (ApMV), ilarviruses. Надеждното откриване на тези вируси се изисква в схемите за сертифициране с цел предоставяне на производители на тестван за вируси посадъчен материал и предотвратяване на тяхното разпространение чрез интродуциран заразен посадъчен материал. През 2019 и 2020 г. са проведени визуални наблюдения и са събрани общо 664 листни проби от няколко разсадника и млади овощни градини, които са тестирани чрез DAS-ELISA метода. Серологично идентифицирани са PDV и PNRSV. Вирусът ApMV не е открит в нито едно от тестираните дръвчета в разсадниците и младите градини. Общият процент на вирусна инфекция е 11.4%. PDV е най-разпространения (7.4%), както в разсадниците (7.2%) така и в младите градини (7.6%), следван от PNRSV 4.1% в разсадниците и 4.0% в новосъздадените градини. Резултатите показват различна степен на разпространение на вирусите в разсадниците и младите овощни градини от 0.9% в черешов разсадник в Пловдив област до 31.6% в едногодишна градина в района на Петрич.

Ключови думи: черешов посадъчен материал, DAS-ELISA, инфекции, PNRSV, PDV, ApMV

INTRODUCTION

Sweet cherry (*Prunus avium* L.) is a traditional and important fruit crop in Bulgaria and its cultivation is favored by the soil, climatic and topographical conditions. The sweet cherry takes the second place after the walnut from all fruit species grown in the country in terms of production area with 54 960 t in 2019 (MAF, 2020).

Cherry trees are known to be hosts for more than 30 viruses and virus diseases (Németh, 1986; Myrta and Savino, 2008). Among them, the ilarvirus species - prunus necrotic ringspot virus (PNRSV), prune dwarf virus (PDV) and apple mosaic virus (ApMV), were reported as the most serious threats for the commercial production of sweet and sour cherry (Pallás et al., 2012). Both, PNRSV and PDV can reduce bud-take in the nurseries, reaching 40 to 50% compared to the healthy stocks in the case of PDV. In addition, both viruses can cause slower growth of young trees (Çağlayan et al., 2011; Németh 1986). Yield reduction and significant losses in sweet cherry trees infected with PNRSV and PDV were reported by Uyemoto and Scott (1992).

ApMV has a worldwide distribution (Diekmann and Putter, 1996). Besides sweet and sour cherry, ApMV infects many other species as hops, rose, hazelnut and apple (Fulton, 1983; Nemeth, 1986).

The symptoms caused by ilarviruses are very similar and may be misleading during preliminary field diagnosis. They may also vary in terms of virus isolate, host species or cultivar, climatic conditions, etc., or lead to latent infection (Pallás et al., 2012).

All three viruses spread through infected propagating material (Mink, 1992). PNRSV and PDV are also seed- and pollen-born and can be spread by pollinating insects (Cole et al., 1982; Hamilton et al., 1984; Kelley and Cameron, 1986). Seed transmission of ApMV in hazelnuts is reported, as well (Cameron and Thompson, 1985).

PNRSV, PDV and ApMV occur often in sweet and sour cherry cultivars, in the rootstocks, and therefore the European and Mediterranean Plant Protection Organization (EPPO) require their testing through a certification scheme (OEPP/EPPO 2001). Their reliable detection is of crucial importance and aimed to provide virus-tested propagation material to the cherry-growing industry and prevent their invasion by imported infected plant materials.

The aim of the present study was to determine the incidence and distribution of ilarviruses in sweet and sour cherry nurseries and 'young' orchards in South Bulgaria.

MATERIALS AND METHODS

Field surveys and plant material

Field observations and leaf samples were collected in the active growing seasons of 2019 and 2020. The surveyed locations were in five major stone fruit-growing regions of South Bulgaria: Kyustendil, Petrich, Plovdiv, Sliven and Kardzhali. In total 664 sweet and sour cherry trees were sampled. They included 390 nursery trees, 364 of which were sampled from sweet cherries from more than 28 cultivars and 26 samples were from sour cherry trees of 3 cultivars. The other 274 leaf samples were collected from five young (1-3 years old) orchards established with propagating material from Greece and Serbia (Table 1). Each leaf sample (4-8 leaves/tree) was taken from the four quadrants of the tree. The samples were collected randomly, from trees both with and without manifestation of virus-like symptoms.

Serological assays

Collected leaf samples were tested for PNRSV, PDV and ApMV by double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELI¬SA) (Clark and Adams, 1977) following the manufacturer's protocol (LOEWE Biochemica GmbH). The photometric measurement was done at 405 nm after 2 h. Samples were considered as positive if their absorbance values were more than three times higher, than the negative control.

RESULTS AND DISCUSSIONS

Field observations

In surveyed nurseries and "young" orchards, over 7,000 cherry trees were inspected for virus symptoms.

Most of the trees did not show any virus-like symptoms. Symptomatic trees showing single leaves with diffused chlorotic rings and/or spots (Figure 1A) were observed only on cv. Early Lory and cv. Van infected with PDV in the two nurseries (Kardzhali and Kyustendil regions).

Virus symptoms were observed in serologically proven PNRSV infected trees grown in 'young' orchards, but many of them were symptomless. Necrotic lesions and spots, giving a tattered appearance of leaves were noted in one orchard (Sliven region), while lighter green spots and chlorosis around the central and secondary veins, with perforation of the tissue were visible on the leaves of the trees grown in two years old orchard in Petrich region. (Figure 1 B and C). Similarly, chlorotic patterns and yellowish ring spots on leaves of sweet cherry trees grown in one 'young' orchard of Kardzhali district infected with PDV were observed.

Often, ilarvirus infections of sweet and sour cherry are symptomless, and when some symptoms are present, they can be easily confused with the symptoms caused by some mineral deficiencies, pesticide damages and/ or genetic disorders. This requires mandatory testing of propagating material to verify its health physiological status.

Detection of ilarviruses by DAS-ELISA

In total, 664 leaf samples from sweet and sour cherry trees from five nurseries and five "young" orchards were serologically analyzed for PNRSV, PDV and ApMV and the results are presented in Table 1. The results from DAS-ELISA tests showed infections with both PNRSV and PDV and absence of ApMV. The results for absence of ApMV are in agreement with that of Borisova et al. (2013), as well with the recently reported results from ELISA tests of 2090 samples from 43 commercial and three collection sweet and sour orchards in six regions of Bulgaria (Kamenova et al., 2020). Unlike our results, a 12% infection rate of sweet cherry with ApMV was found in the Plovdiv region of the country (Milusheva and Zivondov, 2009).

DAS-ELISA revealed that 44 out of 390 nursery tested samples were infected with PDV or PNRSV. The total infection rate was 11.3%, with PDV - 7.2% and with PNRSV - 4.1%. A similar infection level (12.2%) with the two viruses in sweet and sour cherry nurseries in the Czech Republic with much greater infection with PDV (10.2%), than with PNRSV (1.8%) was reported by Suchá and Svobodová (2010). The total infection rate of 10.0% with PNRSV, PDV and ACLSV in sweet cherry nurseries

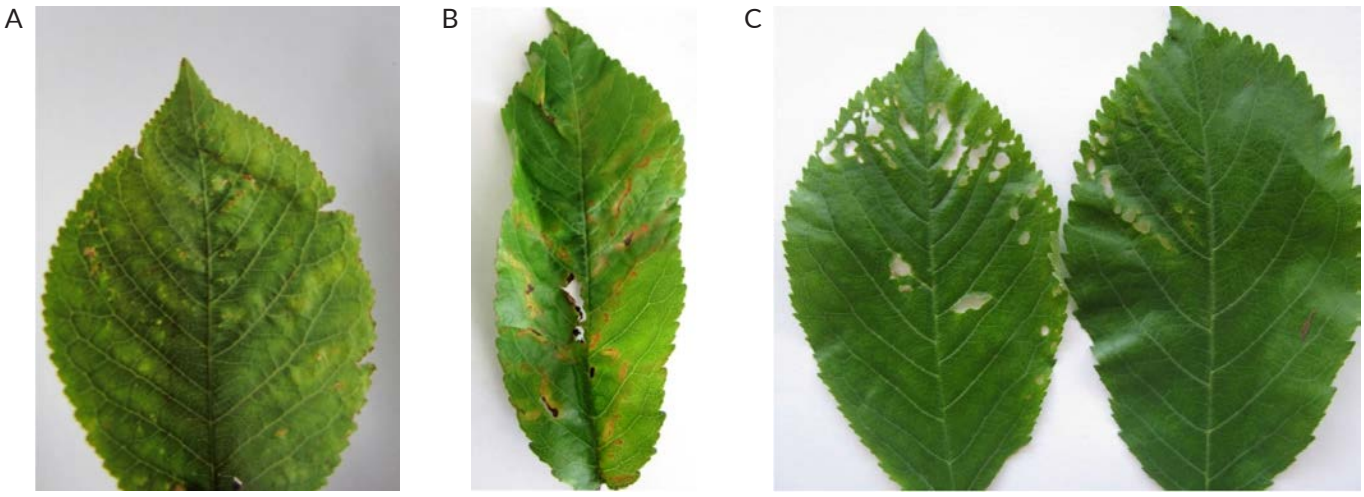

Figure 1. Symptoms on the leaves of sweet cherry trees. A/ chlorotic rings and spots on nursery sweet cherry tree cv. Van infected with PDV; B/ chlorotic and necrotic spots and C/ shot holes on sweet cherry trees grown in "young" orchards infected with PNRSV (original A. Borisova and I. Kamenova)

in Albania also was reported (Musa et al., 2010). Studying the incidence of PNRSV, PDV, ToRSV and PPV in stone fruit nurseries in Chile Guido and Mónica (2002) found an overall infection rate of 7.1% in sweet cherry. PNRSV was detected in 6.0% of tested one-year-old seedlings from sweet cherry nurseries in Jordan (Salem et al., 2003).

Since the fruit trees have been propagated vegetatively, the presence of more than one virus within one tree is very common. In the present study did not find mixed infections of ilarviruses, although such have been reported by our previous researches (Borisova et al., 2013; Kamenova et al., 2020). Due to their similar way of transmission, these viruses often infect the host simultaneously (Guo et al., 1995; Sánchez-Navarro and Pallás, 1997).

Difference in the average ilarviral incidence among the nurseries was ranging from 0.9% to 30.0%. The highest infection rate (30.0%) was found in the sweet cherry nursery located in Vratsa, Kyustendil district, followed by the infection rates of 28.6%, 14.8%, and 5.5% found in Petelovo, Kardzhali district, Vaksevo and Sovolyano, Kyustendil district, respectively. The lowest infection in sweet cherry trees (0.9%) was noted in the nursery of Malak chardak, Plovdiv district while that of sour cherry trees was 5.8%. All tested one-year-old sour cherry seedlings grown in the nursery of Vratsa were free for the tested ilar viruses. Due to the small number of tested samples from sour cherry trees grown in the nurseries, the results for the presence of tested viruses are not representative.

Table 1. DAS-ELISA results of PDV and PNRSV incidence in surveyed nurseries and 'young' orchards in Bulgaria

Region / District	Orchard location / Species		Samples		Positive for:	
			Number tested	Number infected (%)	PNRSV (%)	PDV (%)
South Western / Kyustendil	nursery Vaksevo / sweet cherry		54	8 (14.8)	0	8
South Western / Kyustendil	nursery Sovolyano / sweet cherry		90	5 (5.5)	0	5
South Western / Kyustendil	nursery Vratsa	sweet cherry	70	21 (30.0)	14	7
		sour cherry	19	0	0	0
South East / Kardzhali	nursery Petelovo/ sweet cherry		28	8 (28.6)	0	8
South Central / Plovdiv	nursery Malak chardak	sweet cherry	112	1 (0.9)	1	0
		sour cherry	17	1 (5.8)	1	0
South Western / Kyustendil	'young' orchard Lilyach / sweet cherry		90	9 (10.0)	2	7
South Western / Petrich	'young' orchard Petrich / sweet cherry		39	2 (5.1)	2	0
South Western / Petrich	'young' orchards Kromidovo/ sweet cherry	two years old	65	7 (10.8)	1	6
		one year old	19	6 (31.6)	0	6
South East / Sliven	'young' orchard Panaretovtsi / sweet cherry		61	8 (13.1)	6	2
Total	5 nurseries; 5 'young' orchards		664	76 (11.4)	27 (4.1)	49 (7.4)

Each orchard grown tree consists of the rootstock and the scion cultivar, and sometimes by an interstock cultivar. All of them can be infected and their sensitivity to the virus infection is different. The relative high incidence of PDV and/or PNRSV in some cultivars, as Kyustendilska hrushtyalka (77,0%) and Kozerska (61.0%) from the nursery located in Vratsa, as well in cv. Ferrovia (75.0%) from Vaksevo nursery, in cv. Bing (66.0%) from Sovolyano nursery and in cv. Early Lory (80.0%) from Petelovo nursery suggested that infected scion buds and/or rootstocks were used to propagate the nursery trees. The eradication of infected cultivars and rootstocks is one of the ways to control the spread of pome and stone fruit viruses. It can be applied to individual plants mainly in mother plots and nurseries, but generally, it is not effective over large geographic areas. The eradication involves regular tests of the orchards and the nurseries and the immediate removal of infected trees before further virus spreads (Barba et al., 2015).

The average infection rate of PNRSV and PDV in the 'young' (1-3 years old) commercial orchards established with propagation material imported from Greece and Serbia, was 11.7%. The highest infection (31.6%) was found in the one-year-old orchard located in Petrich district (Kromidovo), followed by the orchard in Sliven district (Panaretovtsi) – 13.1% and the lowest infection (5.1%) was detected in the orchard situated in Petrich. PNRSV and PDV are pollen-borne viruses and the infection occurs during the pollination e.g., when the trees are around 4-5 years old and start to blossom. The ilarvirus infection of surveyed 1-3 years old orchards corresponds to those initially tested at the time of the plantation. The relatively high incidence of PNRSV and PDV in sweet cherries was reported from the neighbouring countries Serbia (Mandic et. al., 2007) and Greece (Maliogka et. al, 2010).

There are no curative treatments to virus and virus-like diseases, including ilarviruses in fruit tree species. The preventive measures, like plant quarantine and certification schemes, are useful tools that can be used to protect or improve the health status of fruit trees. Both are official activities, which remain under the responsibility of the Government. The presence of PNRSV and PDV in sweet and sour cherry nursery trees requires the implementation of certification programs in the production of their propagating material in Bulgaria in order to prevent further spread of the viruses. It is essential for growers to use virus-free certified plants in new orchards or replant orchards.

CONCLUSIONS

The present study represents the first large-scale survey carried out in sweet cherry nurseries and newly established orchards in Bulgaria in regards to ilarvirus pathogens. The overall infection rate was 10.7%. PDV was the most frequent (6.9%), followed by PNRSV (3.8%). ApMV virus was not detected in any of the tested one-year-old seedlings and young trees. This survey confirmed the previous studies on the distributions of the PNRSV and PDV in the main stone fruit-growing areas. However, their presence in the nurseries underlines the importance of their establishment with a well organized and fully implemented certification programme.

The collection of scions and seeds for the production of fruit propagating materials should be performed only mother trees of authentic rootstock and scion cultivars, with an annual check of their viral status.

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