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Detection of Viruses Infecting Sweet Cherry Cultivars and Elites at the Institute of Agriculture-Kyustendil, Bulgaria

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Abstract

To assess the virus infections of new and commercial sweet cherry cultivars and elites, surveys were carried out in two collection orchards at the Institute of Agriculture – Kyustendil located in the Southwest Bulgaria. In the spring of 2012 and 2013 a total of 165 samples were individually collected and tested by DAS-ELISA for *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Plum pox virus* (PPV), *Apple chlorotic leafspot virus* (ACLSV), *Cherry leaf roll virus* (CLRV) and *Raspberry ringspot virus* (RpRSV). A total of 24.2 % of ELISA-tested samples was infected at least by single virus. PDV (13.9 %) was the most prevalent followed by ACLSV (5.4 %) and PNRSV (4.8 %). The other viruses were no presented. The infection was found in both cultivars and elites. These results demonstrated that measures should be taken to avoid using infected parents for crossing.

Keywords: Bulgaria, Kyustendil, sweet cherry, cultivars, viruses, ELISA

Introduction

The sweet cherry (*Prunus avium* (L.)) is the commonest cultivated fruit species represented by 9 799 ha in Bulgaria (Anonymous, 2014) and is one of the most investigated species at the Institute of Agriculture – Kyustendil. The Institute has a rich gene-stock of 185 sweet cherry cultivars and 1100 hybrids. It was selected 59 sweet cherry hybrids as a perspective.

To revealing the full genetic potential of the created hybrids, elites and cultivars it is necessary to monitoring their phytosanitary status, including virus diseases. It is known that healthy plants (free from pathogenic viruses) offer better opportunities in terms of vegetative development, are less susceptible to other diseases and have a higher productivity and fruit quality (Jakubczyk et al., 1997; Bassi and Matelli, 2003).

Cherry trees are known to be hosts for more than 30 viruses and virus diseases (Németh, 1986; Myrta and Savino, 2007). The most common RNA viruses found in sweet cherry include Prunus necrotic ringspot ilarvirus (PNRSV), Prune dwarf ilarvirus (PDV), Apple chlorotic leafspot trichovirus (ACLSV) and Apple mosaic ilarvirus (ApMV) (Myrta et al., 2003; Mandic et al., 2007). Plum pox potyvirus (PPV) was detected in naturally infected trees of sweet cherry in Southern Italy (Crescenzi et al., 1995), in sweet and sour cherry in Bulgaria (Topchiiska, 1996) and in Romania (Maxim et al., 2002).

In this study, we presented results of the detection and distribution of six main stone fruit viruses in two sweet cherry collection orchards at the Institute of Agriculture – Kyustendil, Bulgaria.

Materials and Methods

Plant Material

In two consecutive years 2012–2013, one hundred and sixty-five sweet cherry accessions from 14 cultivars and 24 elites were visually inspected and sampled. All samples were collected from the accessions growing in two collection orchard locates at the Institute of Agriculture – Kyustendil, Bulgaria. The collection orchards were established in 2000 and 2002 respectively. All cultivars and elites were grafted on rootstock *P. mahaleb* and trees were planted at a spacing 6 x 5 m and maintained under the conventional agrotechnical and plant-protection practices. Accessions belonged to different sweet cherry cultivars of different origin, from international exchanges and also from the native elites, which

were selected as promising from hybrids created at the Institute of Agriculture – Kyustendil (Table 1)

Elisa

Leaf samples were collected randomly from different branches around and across the tree early in spring (late April and May). All leaf samples were tested by DAS-ELISA (Clark and Adams, 1977) for the presence of PNRSV (*Prunus necrotic ringspot virus*), PDV (*Prune dwarf virus*)**,** CLRV (*Cherry leaf roll virus*), RpRSV (*Raspberry ringspot virus*) and PPV (*Plum pox virus*) using commercial kits (Loewe, Germany). DAS-ELISA for ACLSV (*Apple chlorotic leafspot virus*) was according to the modification suggested by Flegg and Clark (1979).

Results

Field observations

During visual inspection, most of investigated cultivars and elites showed no symptoms characteristic of viral infection. Virus symptoms were seen in cultivars Kakianes and Vega and consisted of single leaves with diffused chlorotic rings and/or spots (fig.1a) during the spring. It was observed on the leaves of trees of Kakianes infected with PDV also necrotic lesions or spots, which give a tattered appearance of leaves like symptoms caused by PNRSV (fig.1b). There were not symptoms on leaves or fruits of trees of elites № 32/28 infected by PDV, but it was found in all of them dry main branches.

According to the obtained data from the serological analysis the cultivars 'Merchant' was 100% infected with PNRSV, but symptoms of viral infection on leaves, fruits or branches of infected trees were not observed

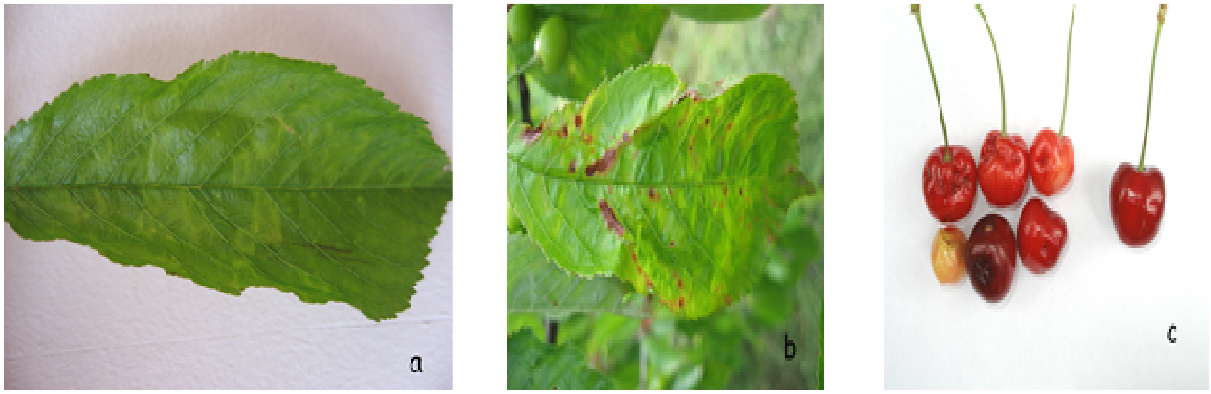

Figure 1. Field symptoms observed in the monitored sweet cherry collection orchards **a**: diffused chlorotic rings on leaves; **b**: necrotic lesions and spots on leaves; **c**: necrosis on fruits

In the field observations in our study were identified symptoms of the virus infection on single fruits of cultivar Kozerska (fig.1c), ELISA tests confirmed the presence of ACLSV.

Virus infection

The results from the serological analysis of the tested trees of investigated cultivars and elites are given in Table 1. Forty out of 165 tested samples reacted to at least one virus, showing an infection rate of 24.2 %. PDV infection was found at the highest rate (13.9 %), followed by ACLSV (5.4 %) and PNRSV (4.8 %). It was not found mixed infections in our investigation.

Table 1. ELISA -testing of virus diseases in two of sweet cherry collection orchards at the Institute of Agriculture – Kyustendil, Bulgaria 2012-2013

Name cultivar or elite	No. of samples		Virus-infected trees, No(%)					
	Tested	Infected,%	PNRSV	PDV	PPV	ACLSV	CLRV	RpRSV
Pobeda Krimska	6	0	0	0	0	0	0	0
Bigarreau Burlat	5	0	0	0	0	0	0	0
Johana	4	0	0	0	0	0	0	0
Vanda	4	0	0	0	0	0	0	0
Van	5	1	0	1	0	0	0	0
Bing	5	0	0	0	0	0	0	0
Vega	9	8	0	8	0	0	0	0
Kozerska	4	4	0	0	0	4	0	0
Valeska	5	2	0	0	0	2	0	0
Regina	**7**	3	0	0	0	3	0	0
Merchant	8	8	**8**	0	0	0	0	0
Tragana Edesis	3	0	0	0	0	0	0	0
Kakianes	4	2	0	**2**	0	0	0	0
Stela	3	0	0	0	0	0	0	0
№ 6371	3	0	0	0	0	0	0	0
№ 6400	9	0	0	0	0	0	0	0
№ 5750	3	0	0	0	0	0	0	0
№ 3456	4	1	0	1	0	0	0	0
№ 6383	3	0	0	0	0	0	0	0
№ 3419	3	0	0	0	0	0	0	0
№ 5938	4	1	0	1	0	0	0	0
№ 6351	3	0	0	0	0	0	0	0
№ 3225	2	2	0	2	0	0	0	0
№ 5937	4	0	0	0	0	0	0	0
№ 5752	3	0	0	0	0	0	0	0
№ 1890	3	0	0	0	0	0	0	0
№ 6387	5	0	0	0	0	0	0	0
№ 3353	2	2	0	2	0	0	0	0
№ 65/46	5	0	0	0	0	0	0	0
№ 13791	3	0	0	0	0	0	0	0
№ 6401	3	0	0	0	0	0	0	0
№ 6541	5	0	0	0	0	0	0	0
№ 32/28	5	5	0	5	0	0	0	0
№ 6374	4	0	0	0	0	0	0	0
№ 3220	5	0	0	0	0	0	0	0
№ 6528	3	0	0	0	0	0	0	0
№ 3227	5	1	0	1	0	0	0	0
№ 1539	4	0	0	0	0	0	0	0
TOTAL	**165**	**40(24.0)**	**8(4.8)**	**23(13.9)**	**0**	**9(5.4)**	**0**	**0**

No PPV, CLRV and RpRSV infected tree was detected from any tested samples (Tab. 1).

According to the obtained data the cultivar 'Merchant' was 100% infected with PNRSV and cultivar 'Kozerska' was 100 % infected with ACLSV. Among the infected cultivars the percentage of ACLSV in cultivar

'Valeska' and 'Regina' was 40 % and 42.8 %, respectively. The PDV infection was found in both cultivars and elites. The infection level of PDV was 100 % in elite № 3225, № 3353 and № 32/28, 88.8 % in cultivar Vega, 55.5 % in Kakianes, 25 % in elite № 3456 and № 5938, 20 % in cultivar Van and elite № 3227.

Discussion

In general, symptoms of PNRSV appear in the first year after infection (acute or shock stage), and then commonly become symptomless like of our case, although some strains cause recurrent symptoms annually (Nyland et al., 1976; Wells and Kirkpatrick, 1986). Most strains of ACLSV are latent in sweet cherry trees, others can be responsible for necrosis on fruits of susceptible sweet cherry cultivars (Desvignes and Boye, 1989; Desvignes, 1999), like in our investigation.

The results on detection of viruses infecting sweet cherry are similar with those obtained from our previous investigation and from studies in other countries. The results obtained of study on the agents of viral diseases of stone fruit species growing in Kyustendil region during the period 2000 - 2012 showed that 22.6% of 751 tested sweet cherry trees were infected at least by single virus and highest rate of viral infection was found of PDV – 16.0 %, followed by PNRSV (4.0 %) and ACLSV (2.6%) (Borisova et al, 2013).

The extended field studies carried out during 1992 to 2007 in the Mediterranean region, involved 14 countries demonstrated a high incidence (23.5%) of ilarvirus infection. Prevailing single viruse in cherry was PDV (35.4% of the total tested or 71% of the infected trees) (Pallas et al., 2012).

Recently a large number of sweet and sour cherry samples collected from several regions of Bulgaria were tested serologicaly (with strain-specific monoclonal antibodies) and molecularly (with strain-specific primers) (Kamenova et al., 2013) and no infection of PPV was detected.

One of the main reasons for identification of PDV in some of studied elites was using infected parents for crossing. PNRSV and PDV are reported to be pollen and seed transmitted (Kelley and Cameron, 1986; Amari et al., 2004). Their presence in collections orchards used as source materials for genetic breeding (pollen or seeds) make urgent requirement to establish appropriate protocols to exclude, or otherwise check them to avoid the diffusion with propagating material

Conclusions

A total of 24.2 % of tested sweet cherry cultivars and elites was infected by viruses. PDV was the most detected virus (13.9 %), followed by ACLSV (5.4 %) and PNRSV (4.8 %). No PPV, CLRV and RpRSV infection was detected.

All trees of cultivars Pobeda Krimska, Bigarreau Burlat, Johana, Vanda, Bing, Tragana Edesis and Stela and 10 of investigated elites were free of all tested viruses.

Researchers from different but undoubtedly complementary scientific disciplines like geneticians and virologists must be work in co-operation to avoid viral infections during the process of genetic breeding.

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