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VIRUS STATUS OF NEW BRED CHERRY CULTIVARS AND ELITES

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Abstract

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The aim of the study was to find out the virus status of 2 new bred cherry cultivars and 10 cherry elites. Visual inspections for symptoms presence/absence and enzyme-linked immunosorbent assay (ELISA) were carried out. 55 trees were analysed serologically, as 24 ones of them were tested in two consecutive vegetative cycles. In order to find out the influence of *Cherry leaf roll virus* on the vegetative and reproductive behaviors, the yield and the mean terminal shoot length of infected and uninfected trees of one elite were measured and compared. The data showed that the most distributed virus was *Cherry leaf roll virus* (CLRV), identified in single or mixed infection in 56.4 % of the tested trees. The infection levels for other phytopathogens tested were as follows: *Apple chlorotic leaf* spot virus (ACLSV) – 16.4%, *Arabis mosaic virus* (ArMV) – 9%, *Prune dwarf virus* (PDV) - 7.3 % and *Prunus necrotic ring spot virus* (PNRSV) – 5.4%. *Raspberry ring spot virus* (RpRSV), *Plum pox virus* (PPV) and *Petunia asteroid mosaic virus* (PeAMV) were not detected in the samples analysed. The data about the yield and the mean shoot length showed that in some cases the yield of the diseased trees was two times less in comparison with the healthy ones and the mean shoot growth was vastly decreased.

Key words: sweet cherry, elites, virus status, serological detection

Introduction

The breeding programme for establishing of new sweet cherry cultivars in Fruit Growing Institute (FGI), Plovdiv has started since 1989 (Zhivondov, 1994; Zhivondov et al., 2004; Zhivondov, 2009; Zhivondov and Gercheva, 2009). An abundant F1 hybrid fund was developed in FGI as the breeding values were evaluated for some cultivars and their progenies as donors of worth economic traits (Zhivondov et al., 2000; Zhivondov et al., 2004). The first results of the breeding programme were the new cultivars Kossara (Zhivondov and Gercheva, 2009), Rosita (Zhivondov and Gercheva, 2010) and Rozalina (Zhivondov, 2011), officially acknowledged by the Executive Agency for Variety Testing, Field Inspection and Seed Control.

A large number of hybrids and elites have been in advanced stage (2nd and 3rd) of evaluation. The negative economic effect of the virus diseases on sweet cherry finds expression in decline of leaf and flower buds, forming of less fruits and poor flavour, decreasing of tree vigour and their premature dying. Besides the yield of fruit-bearing orchards, the virus infections could compromise the breeding of new cultivars, as in selection the virus contaminations would reflect on the evaluation of the economic traits of the elites and hybrids.

The aim of the study was to find out the virus status of the new bred cherry cultivars, Kossara and Rozalina and 10 cherry elites. The yield losses of 'Van' cultivar and one elite infected by CLRV were evaluated, too.

Materials and Methods

The study included the new bred cultivars Kossara and Rozalina, 10 cherry elites (Table 1) and standard cultivars Bigarreau Burlat, Bing and Van. Each of experimental genotypes was grafted onto the rootstocks 'Gilela 5', *Prunus mahaleb* and *Prunus avium.*

Virological investigations included visual inspection for presence of virus-like symptoms and tests by enzyme - linked immunosorbent assay (ELISA). 55 trees were analysed by ELISA, as 24 of them in 2 vegetative cycles. The samples were collected from symptomatic trees as well as

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Table 1
Origin of the studied cultivars and elites

Parents	Cultivar / Elite
`Bigarreau Burlat` x `Ranna Černa`	Kossara
`Van` o. p.	Rozalina
`Compact Stella` o. p.	El. 8-65
`Van` o. p.	El. 17-31
`Van` o. p.	El. 17-37
`Van` o. p.	El. 17-44
`Van` o. p.	El. 17-90
`Van` o. p.	El. 17-92
`Van` o. p.	El. 17-136
`Van` o. p.	El. 20-31
`Van` o. p.	El. 20-47
`Starcrimson cherry` o. p.	El. 20-77

o. p. - open polination

from symptomless ones. The collected samples were tested for 8 sap transmissible viruses: from *Ilarvirus*, *Prunus necrotic ring spot virus* (PNRSV) and *Prune dwarf virus* (PDV); from *Trichovirus*, *Apple chlorotic leaf spot virus* (ACLSV); from *Tombusvirus*, *Petunia asteroid mosaic virus* (PeAMV); from *Potyvirus, Plum pox virus* (PPV) and from *Nepovirus*, *Cherry leaf roll virus* (CLRV), *Arabis mosaic virus* (ArMV) and *Raspberry ring spot virus* (RpRSV). Two ELISA variants were performed – for the identification of PNRSV, PDV, PPV, CLRV, ArMV, RpRSV и PeAMV was applied the variant double antibody sandwich (Clark and Adams, 1977) and for detection of ACLSV, the test was carried out according to modified procedure named cocktail ELISA developed by Flegg and Clark, (1979). The commercial diagnostics set manufactured by Sanofi Diagnostics Pasteur, Bioreba AG и Loewe Phytodiagnostica GmbH were applied.

The yield and the mean terminal shoot length of some elites were measured for each individual tree in order to find out the influence of the virus infections on both vegetative and reproductive behaviour of the plants. The yield was determinate by weighting of fruit quantity per tree and the terminal shoot elongation rate was calculated by the mean length of one-year shoots of control branches.

Results

In the spring, the following symptoms were observed: chlorotic spots, deformation, rolling of the leaves; shortened internodes of the shoots and drying of some of branches; smaller fruits incompletely coloured, sometimes with distorted shape and in some cases with necrotic spots.

The data from ELISA (Table 2) showed that the most distributed was CLRV identified in single (38.2 %) or mixed (18.2 %) infection in 56.4 % of tested samples (Tables 2 and 3). The levels of infection in other studied viruses were as follows: ACLSV – 16.4 %, ArMV – 9%, PDV – 7.3 %, PNRSV – 5.4 %. RpRSV, PPV and PeAMV were not detected in the analysed samples.

In some cases the studied viruses were diagnosed in mixed infection. The most frequent virus combinations were CLRV + ACLSV, CLRV + PDV, CLRV + PNRSV.

The data about the yield and the mean shoot length revealed that in the same climate conditions, in some cases the yield of the diseased trees was two times less in comparison with the healthy ones (Tables 4 and 5) and the one-year growth was four times decreased (Table 5).

Discussion

Until recently CLRV was considered for a less distributed in sweet cherry in our country. In our previous investigation on virus status of cherry cultivars, the virus was also detected in the highest level – 32% (Milusheva and Zhivondov, 2009). According to the results from the cited study ACLCV and ArMV were identified in 15% and 13% respectively, *i.e.* the levels were similar to the data from the current study. CLRV and ArMV belong to genus *Nepovirus*. In the nature, both viruses are transmitted by pollen, seeds and nematodes from genus *Xiphinema* and *Longidorus* and that favours the incidence of the pathogens in the orchards as well as in the neighbouring plantations. That means of epidemiology of the *Nepovirus* could explain the high level of CLRV infection. It was found out that the infected pollen has poor fertility and the seeds obtained from diseased trees have decreased germination (Nemeth, 1986). The second distributed virus ACLSV infects the trees latently but in some susceptible cultivars, in single or in mixed infection with PNRSV, can cause necrosis on cherry fruits. ACLSV is transmitted mainly by propagating material and up to now the vectors in nature are unknown. This means that it is necessary to control the production of planting material of elites deriving from elites as well as rootstocks (Sertkaya, 2008).

The viruses belonging to *Ilarvirus,* PNRSV and PDV were detected in relative low percentage, respectively in 5.4% and 7.3% of tested samples. Both viruses are also transmitted by pollen and seeds and in mixed infection with CLRV cause destruction of the trees for a period of 3 to 4 years.

The mixed virus infections can often be detected under natural infection background and in many cases they lead to

stunt of growth, fruitless, partially or completely dying of the trees and thus the losses may increase (Nemeth, 1986).

In the process of the study, trees with stunt growth, viral symptoms on the leaves, little and poor quality fruits were observed but in some cases it was impossible to identified any virus by ELISA, therefore it might concern of an infection caused by virus (or viruses), which cannot be detected serologically and it is necessary to apply other detection methods.

Conclusions

The result obtained clearly showed an increase of the infection with CLRV of some cherry cultivars in the country.

As a result of CLRV infection, the yield of the diseased cherry trees of reduced vastly and the termal shoot length decreased several times. This indicates that the expansion of CLRV infection in Bulgaria could have significant economical importance.

Table 2
Results from ELISA of cherry cultivars and elites

Cultivar	Rootstock	PNRSV	PDV	ACLSV	PPV	CLRV	ArMV	RpRSV	PeAMV
		n/N	n/N	n/N	n/N	n/N	n/N	n/N	n/N
`Kossara`	`Gisela 5`	0/6	0/6	1t/6	0/6	5t/6	2t/6	0/6	0/6
`Kossara`	*P.mahaleb*	0/1	0/1	1t/1	0/1	1t/1	0/1	0/1	0/1
`Rozalina`	`Gisela 5`	0/2	0/2	1t/2	0/2	0/2	0/2	0/2	0/2
`Rozalina`	*P.mahaleb*	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
`Rozalina`	*P. avium*	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
`B.Burlat`	*P.mahaleb*	0/3	0/3	0/3	0/3	2t/3	0/3	0/3	0/3
`Bing`	*P.mahaleb*	0/1	0/1	0/1	0/1	1t/1	0/1	0/1	0/1
`Bing`	*P. avium*	0/1	1t/1	0/1	0/1	1t/1	0/1	0/1	0/1
`Van`	`Gisela 5`	0/3	0/3	1t/3	0/3	1t/3	0/3	0/3	0/3
`Van`	*P. avium*	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
El.8-65	`Gisela 5`	0/1	0/1	0/1	0/1	1t/1	1t/1	0/1	0/1
El.8-65	*P.mahaleb*	0/1	1t/1	0/1	0/1	1t/1	0/1	0/1	0/1
El.17-31	`Gisela 5`	0/2	0/2	0/2	0/2	1t/2	0/2	0/2	0/2
El.17-31	*P.mahaleb*	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
El.17-31	*P. avium*	0/4	0/4	0/4	0/4	2t/4	0/4	0/4	0/4
El.17-37	*P.mahaleb*	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
El.17-44	*P.mahaleb*	0/2	1t/2	0/2	0/2	1t/2	0/2	0/2	0/2
El.17-90	`Gisela 5`	0/1	0/1	0/1	0/1	1t/1	0/1	0/1	0/1
El.17-92	*P.mahaleb*	1t/1	0/1	0/1	0/1	1t/1	0/1	0/1	0/1
El.17-136	`Gisela 5`	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
El.17-136	*P.mahaleb*	0/1	0/1	1t/1	0/1	1t/1	0/1	0/1	0/1
El.20-31	`Gisela 5`	1t/3	0/3	1t/3	0/3	3t/3	1t/3	0/3	0/3
El.20-31	*P.mahaleb*	0/1	0/1	0/1	0/1	1t/1	0/1	0/1	0/1
El.20-47	`Gisela 5`	0/3	0/3	1t/3	0/3	1t/3	0/3	0/3	0/3
El.20-47	*P. avium*	0/2	1t/2	1t/2	0/2	1t/2	1t/2	0/2	0/2
El.20-77	`Gisela 5`	1t/2	0/2	0/2	0/2	1t/2	0/2	0/2	0/2
El.20-77	*P.mahaleb*	0/2	0/2	0/2	0/2	2t/2	0/2	0/2	0/2
El.20-77	*P. avium*	0/3	0/3	1t/3	0/3	2t/3	0/3	0/3	0/3
Total		3t/55	4t/55	9t/55	0/55	31t//55	5t/55	0/55	0/55
%		5.45	7.3	16.4	0	56.4	9.09	0	0

N – total number of analysed trees

n – number of trees, reacted positively with antiserum for the given virus

t - tree(s)

Table 3
Rations of CLRV single and mixed infection

Cultivar	Rootstock	N	CLRV positive samples	
			Single infection	Mixed infection
`Kossara`	`Gisela 5`	6	4	2
`Kossara`	*P.mahaleb*	1	0	1
`B.Burlat`	*P.mahaleb*	3	2	0
`Bing`	*P.mahaleb*	1	1	0
`Bing`	*P. avium*	1	0	1
`Van`	`Gisela 5`	3	1	0
El.8-65	`Gisela 5`	1	0	1
El.8-65	*P.mahaleb*	1	0	1
El.17-31	`Gisela 5`	2	1	0
El.17-31	*P. avium*	4	2	0
El.17-44	*P.mahaleb*	2	1	0
El.17-90	`Gisela 5`	1	1	0
El.17-92	*P.mahaleb*	1	1	0
El.17-136	*P.mahaleb*	1	0	1
El.20-31	`Gisela 5`	3	2	1
El.20-31	*P.mahaleb*	1	1	0
El.20-47	`Gisela 5`	3	1	0
El.20-47	*P. avium*	2	0	1
El.20-77	`Gisela 5`	2	1	0
El.20-77	*P.mahaleb*	2	2	0
El.20-77	*P. avium*	3	1	1
Total		55	21	10
%			38.2	18.2

N – total number of analysed trees

Table 4
Yield (kg) data from infected nd uninfected trees of `Van`

№ of tree	Cultivar	Rootstock	Virus status 2010	Yield (kg) 2010
2r-2t	`Van`	`Gisela 5`	ACLSV	2.8
2r-3t	`Van`	`Gisela 5`	CLRV	2.6
2r-3t	`Van`	`Gisela 5`	nv	4.2

nv- no virus identified; r - row; t – tree

Table 5
Yield (kg) and mean shoot length (cm) data from infected and uninfected trees of elite 17-31 in two subsequent vegetative cycles (2009 - 2010)

№ of tree	Elite	Rootstock	VS ‘09	VS’ 10	Yield (кg) ‘09	Yield (кg) ‘10	MSL (cm) ‘09	MSL (cm) ‘10
1r-40t	El.17-31	`Gisela 5`	nv	nv	7.65	14	9.47	13.11
1r-41t	El.17-31	`Gisela 5`	CLRV	CLRV	nd	6.5	4.29	3.62
5r-10t	El.17-31	*P. avium*	CLRV	CLRV	nd	1.2	17.36	4.87
5r-12t	El.17-31	*P. avium*	nv	nv	3.18	7.4	40.79	20.62

MSL - mean shoot length; nd – no data available; nv - no virus identified
r - row; t – tree; VS - virus status

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