ANALYSIS OF AUTOCHTHONOUS PLUM GENOTYPES (*PRUNUS DOMESTICA* L.) IN CROATIA FOR THE PRESENCE OF *PLUM POX VIRUS*

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SUMMARY

Plum pox virus (PPV) is increasingly endangering plum production in Croatia. This study aimed at determining the presence of this virus in autochthonous plum genotypes and at identifying plum trees of particular genotypes, not infected by it. To sort out the broad array of synonyms used in plum variety naming, the samples were submitted to genetic analysis, for defining their affiliation to a particular genotype (variety). In addition, the presence and frequency was assessed of *Apple chlorotic leaf spot virus* (ACLSV) and *Prunus necrotic ringspot virus* (PNRSV) infections in autochthonous plum genotypes. In 2005, leaf and fruit samples from 54 trees were collected in seven Croatian counties, Osjecko-baranjska, Vukovarsko-srijemska, Pozesko-slavonska, Brodsko-posavska, Viroviticko-podravska, Sisacko-moslavacka and Licko-senjska. The variety Bistrica is cultivated in all seven areas while the other cultivars (Bela staroverska, Bijela rana, Bjelica jajara, Brdaklija, Cericanka, Crnica, Dabinka, Debeljara, Glodara, Kalaca, Kamenjara, Komocanka, Mandalenka, Miholjcanka, Pasjara, Pintara, Plavica, Ruzica, Sitna bijelica, Torgulja, Ilinjaca, Trnovaca and Valpovka) were found in particular locations of specific counties. Serological tests were used for establishing the presence of the three viruses. PPV was detected in four plum genotypes, three of which (cv. Glodara from Dolci, cv. Pasjara from Viljevo and cv. Bistrica from Podvrsko) contained the virus in both leaves and fruits, while cv. Glodara from Crkvari contained PPV only in the leaves. All the other plum genotypes tested were found to be PPV-free. PNRSV was detected in two genotypes. It was found both in the leaves and fruits of cv. Komocanka from Caglin, while only the leaves of cv. Debeljara from Batina were infected by this virus. None of the tested plum accessions was infected by ACLSV. Likewise, none of the three viruses was detected in 48 of 54 trees investigated.

Key words: autochthonous plum genotypes, ELISA, PPV, ACLSV, PNRSV, survey.

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INTRODUCTION

Plum production is traditionally one of the major sectors of stone fruit industry in Croatia, plum being the most represented (in excess of 6 million trees) species in the country (Anonymous, 2004). In the continental part of Croatia, i.e. Slavonija and Lika, there are many plum genotypes. Since each farm house traditionally owns at least several plum trees, there is a pronounced interest in preserving autochthonous germplasm. Because domesticated plum types are widespread and have different local names, some of which may be synonyms, it is necessary to differentiate them genetically for proper identification. As a follow-up, it appears desirable to range plum types according to their economic potentiality and health status. Similar studies have already been done in countries surrounding Croatia (Nenadovic-Mratinic, 2000).

Plum pox virus (PPV) is one of the most dangerous fruit tree viruses as it causes both yield losses due to the significant reduction of fruit quality and quantity (Josifovic, 1937) and a rapid decline of infected plum trees, which constitute a natural habitat for the aphid vectors of the virus (Kunze and Krczal, 1971). PPV was first recorded in Croatia in Nova Gradiska (Nikolic, 1949) but is now widespread in almost all regions of the country, where it causes economical damages. This has prompted the evaluation of the impact of PPV infections to autochthonous plum genotypes. In the course of the survey, the occurrence of *Apple chlorotic leaf spot virus* (ACLSV) and *Prunus necrotic ringspot virus* (PNRSV) was also checked in the tested trees. Thus, the present paper provides relevant data to validate the ultimate objective of our study consisting of the identification of healthy propagation material of autochthonous plum genotypes.

MATERIALS AND METHODS

The first information on "mother trees", distribution of plums and their local names (folk names) was collected in cooperation with the "Domestic Plum Brandy Association-Slavonka" from Osijek. We made a list of plum genotypes considered as potential candidates for

mother plants with data about their geographical distribution (Fig. 1). The criteria used for selection of "mother trees" were: good vegetative condition, high yield and absence of evident symptoms. The age of "mother trees" sampled ranged from 8 to 40 years. Trees were selected and marked in May 2005 and each of them was localized using GPS technology (e.g. geographic latitude and longitude, altitude) with an accuracy of 4 meters. Every tree was described according to DUS protocol (CPVO, 2003). For sampling, four shoots were taken from the quadrant of the canopy. Leaf samples were taken 10 to 20 cm from the tip of the shoot, were stored in marked bags in a mobile refrigerator, then frozen at -18°C for 6 h after collection. Ripe fruits samples were collected in succession in July, August and September 2005, according to the maturation rate of each cultivar. Three fruit samples were collected from the quadrant of the canopy of each genotype. Transport and storage procedures were similar to those used for leaf samples.

Ninety-three leaf and fruit samples from 54 plum trees were collected in seven Croatian counties: Osjecko-baranjska, Vukovarsko-srijemska, Pozesko-slavonska, Brodsko-posavska, Viroviticko-podravska, Sisacko-moslavacka and Licko-senjska. Genotype Bistrica was present in all surveyed counties, while the distribution of other plum types varied from location to location. The names of analyzed plum genotypes were: Bela staroverska, Bijela rana, Bjelica jajara, Brdaklija, Cericanka, Crnica, Dabinka, Debeljara, Glodara, Ilinjaca, Kalaca, Kamenjara, Komocanka, Mandalenka, Miholjcanka, Pasjara, Pintara, Plavica, Ruzica, Sitna bjelica, Torgulja, Trnovaca, and Valpovka.

Fig. 1. Locations object of the survey (black dots). Arrows point to locations, where plum trees were infected by both PPV and PNRSV.

Leaf and ripe fruit peel extracts were tested for PPV and PNRSV by DAS-ELISA (Clark and Adams, 1977; Adams, 1978; Cambra *et al.*, 2002) and for ACLSV by DAS-simultaneous ELISA (cocktail-ELISA) (Flegg and Clark, 1979), using commercial kits (Loewe Biochemica, Germany). Absorbance was measured using a Micro Reader I (Hyperion, USA) and samples with the OD values three-fold higher than the mean value of healthy controls were considered as positive.

RESULTS AND DISCUSSION

The majority of the 93 leaf and fruit samples were not visibly affected, except for certain genotypes that exhibited typical symptoms of PPV infection either on the fruits or leaves (Fig. 2). ELISA testing for the presence of PPV, PNRSV and ACLSV in leaf and fruit samples showed that PPV was found in four plum genotypes collected in different locations (Table 1).

Morphological changes of fruits and leaves, i.e. concentric pale green rings, were visible in cv. Bistrica from Podvrsko (Fig. 2A and B) where PPV was detected in extracts from both organs.

PPV-induced symptoms can vary in different genotypes (Festic, 1979). They may not be clearly visible on immature fruits but become evident at ripening in the form of wrinkles, pits and cavities of different size on the surface of infected fruits (Fig. 2B). Fruits form infected plants ripen two to three weeks in advance (Festic, 1979) and have no commercial value. In our investigation, PPV was detected in four plum genotypes, in three of which the virus occurred in both leaves and mature fruits (cv. Glodara from Dolci, cv. Pasjara from Viljevo, cv. Bistrica from Podvrsko). In cv. Glodara from Crkvari, PPV was only detected in leaf extracts.

Adams (1978) reported that PPV detection through standard ELISA in young, hard, green fruit was relevant. We detected that measured absorbance values (A_{405}) were five-fold higher in leaf than in skin extracts from immature fruits and that absorbance values from fruits with visible symptoms were four times higher than those obtained from fruits without symptoms (not shown).

PNRSV was found in three samples belonging to two plum genotypes (Table 1). In cv. Komocanka from Caglin, PNRSV was found in both leaves and mature fruits, while in cv. Debeljara from Batina, it was only present in the leaves. The leaves of PPV-infected cv. Glodara from Crkvari showed mild chlorotic stripes along the veins and near the margin (Fig. 2C). There were no apparent symptoms of PPV infection on plum fruits (Fig. 2D) and the virus was not detected by ELISA. Strikinglys while sampling leaves and fruits of genotypes Glodara from location Dolci and Pasjara from Viljevo that had formerly tested positive for PPV, no typical symptoms of PPV infection were detected. Moreover, no

trace of ACLSV was detected in the tested samples. We have to emphasize that results of performed ELISA were probably influenced by higher average daily temperatures during the summer that lowered virus concentration in plant tissue (Glasa *et al.*, 2003).

This is a preliminary investigation for establishing the occurrence and geographic distribution of PPV, ACLSV and PNRSV in autochthonous plum genotypes in Croatia.

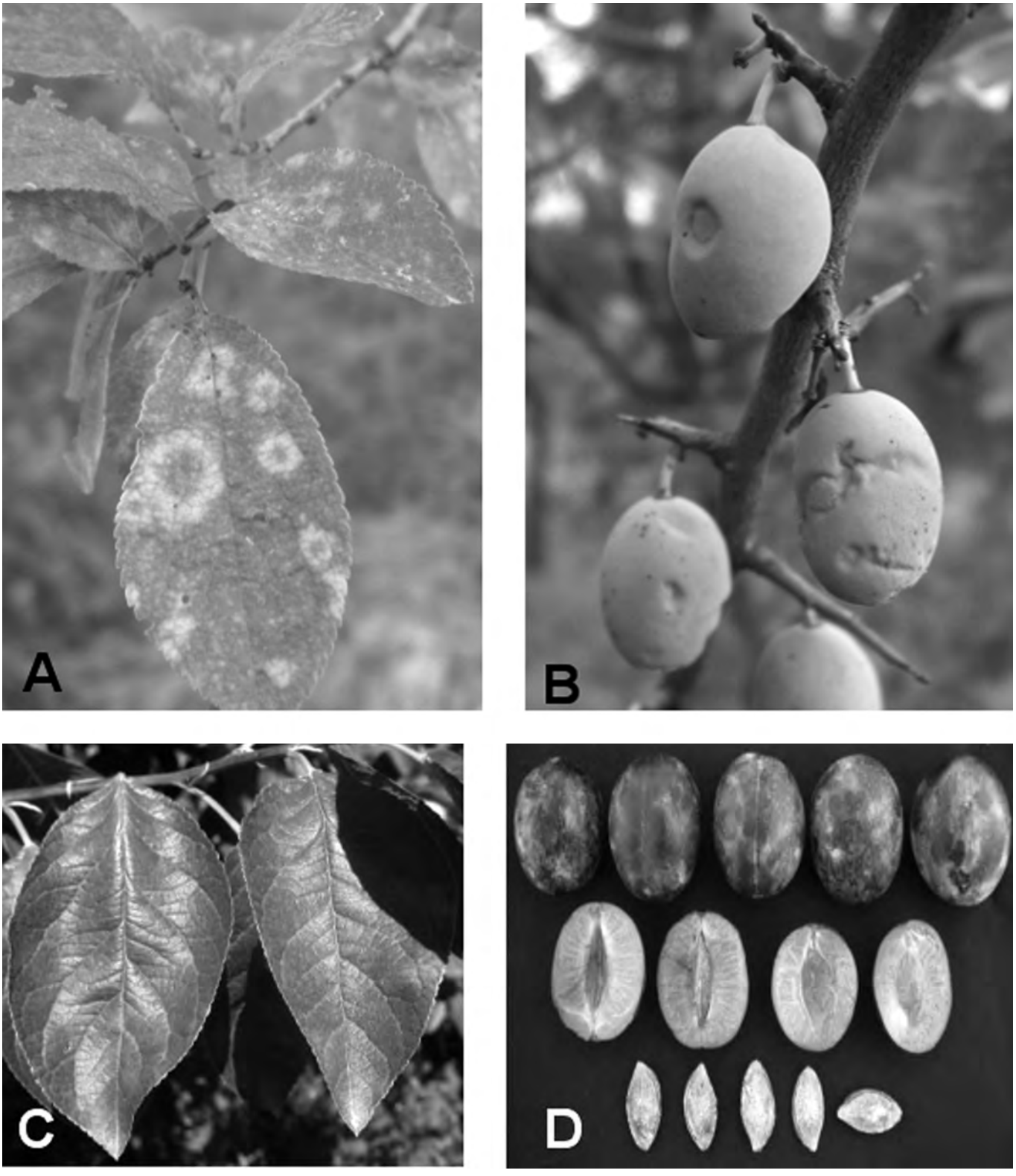

Fig. 2. Symptoms on fruits and leaves of genotype Bistrica from Podvrsko (A and B). Plum genotype Glodara from Crkvari with mild chlorosis along the leaf veins and near the leaf margin (C). Symptomless fruits from the same tree (D).

Table 1. Results of ELISA detection of *Plum pox virus* (PPV), *Prunus necrotic ringspot virus* (PNRSV) and *Apple chlorotic leaf spot virus* (ACLSV) in autochthonous plum genotypes.

No	Cultivar	Locality	Plant organ[(a)]	Tested viruses		
				PPV	PNRSV	ACLSV
1	Turkinia	Cetingrad	L	-	-	-
2	Primorka	Brinje	L	-	-	-
3	Bistrica	Brinje	L	-	-	-
4	Bjelica	Brinje	L	-	-	-
5	Bistrica	Brinje	L	-	-	-
6	Bistrica	Brinje	L	-	-	-
7	Bistrica	Brinje	L	-	-	-
8	Torgulja	Jazavica	L/F	-	-	-
9	Trnovaca	Valpovacka Satnica	L/F	-	-	-
10	Bistrica	Beli Manastir	L/F	-	-	-
11	Komocanka	Caglin	L	-	+	-
			F	-	+	-
12	Glodara	Crkvari	L	+	-	-
			F	-	-	-
13	Torgulja	Nova Josava	L/F	-	-	-
14	Plavica	Caglin	L	-	-	-
15	Bistrica	Sovski dol	L/F	-	-	-
16	Glodara	Dolci	L	+	-	-
			F	+	-	-
17	Torgulja	Valpovacka Satnica	L	-	-	-
18	Cericanka	Andrijasevci	L/F	-	-	-
19	Pasjara	Viljevo	L	+	-	-
			F	+	-	-
20	Cericanka	Cerna-Jakobovci	L/F	-	-	-
21	Cericanka	Cerna	L/F	-	-	-
22	Dabinka	Levanjska Varos	L/F	-	-	-
23	Valpovka	Ivanovci-Valpovo	L/F	-	-	-
24	Valpovka	Valpovo	L/F	-	-	-
25	Valpovka	Valpovo	L/F	-	-	-
26	Bijela rana	Batina	L/F	-	-	-
27	Pintara	Stari Perkovci	L/F	-	-	-
28	Pintara	Vrpolje	L/F	-	-	-
29	Miholjcanka	Viljevo	L/F	-	-	-
30	Mandalenka	Viljevo	L/F	-	-	-
31	Sitna bjelica	Levanjska Varos	L/F	-	-	-
32	Kalaca	Trpinja	L	-	-	-
33	Bijela sitna	Svetoblazje	L/F	-	-	-
34	Bijela sitna	Svetoblazje	L/F	-	-	-
35	Bjelica jajara	Omanovac	L/F	-	-	-
36	Brdaklija	Prekopakra	L	-	-	-
37	Bela staroveska	Viljevo	L/F	-	-	-
38	Torgulja	Valpovo	L/F	-	-	-
39	Debeljara	Batina	L	-	+	-
			F	-	-	-
40	Crnica	Svetoblazje	L/F	-	-	-
41	Trnovaca	Podvrsko	L/F	-	-	-
42	Kamenjara	Prekopakra	L/F	-	-	-
43	Bijela sitna	Gorjani	L/F	-	-	-
44	Bijela sitna	Sovsko Dol	L/F	-	-	-
45	Bistrica	Levanjska Varos	L/F	-	-	-
46	Bijela sitna	Blacko	L	-	-	-
47	Bijela mirisava	Podgradje	L	-	-	-
48	Bijela mirisava	Nijemci	L	-	-	-
49	Crnica	Prekopakra	L	-	-	-
50	Debeljara	Prekopakra	L/F	-	-	-
51	Bjelica	Vocarica	L/F	-	-	-
52	Ruzica	Vocarica	L/F	-	-	-
53	Bistrica	Podvrsko	L	+	-	-
			F	+	-	-
54	Bistrica	Sesvete	L/F	-	-	-

[(a)] L = leaf, F = fruit

In subsequent studies, selected genotypes with desirable morphological and technological characteristics will be tested for the presence of other pathogens that are important for certification (EPPO, 2000).

The present study has shown the potential of the different plum genotypes (cultivars) taken into consideration and the validity of the experimental methods for their authentication. Through this part of this complex survey, which is the first systematic investigation on autochthonous Croatian plum genotypes, we have ascertained their health status with reference to PPV, PNRSV and ACLSV, three major viruses of *Prunus* and have identified the genotypes that can potentially be used as mother plants.

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