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Incidence of virus infections in grapevines from Croatian collection plantations

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Summary. Evaluation of the incidence of virus infections was conducted in two grapevine collection fields. The first was the National collection of Croatian native grapevine cultivars, situated at the "Jazbina" experimentation station in eastern Zagreb, where more than 120 different autochthonous grapevine cultivars are held, collected from different Croatian vine-growing regions. The second was a regional collection located in Risika, on the island of Krk (the North Adriatic region) containing 19 native cultivars from that region. During February 2009 from both collections, 95 plants were selected and tested for presence of eight viruses by ELISA, including: *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Grapevine fleck virus* (GFkV), *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 2* (GLRaV-2), *Grapevine leafroll-associated virus 3* (GLRaV-3), *Grapevine virus A* (GVA) and *Grapevine virus B* (GVB). The dominant virus in both collections was GLRaV-3, present in 75 vines (78.9%) in the National collection and in 73 vines (76.8%) in the Risika collection. The second most frequent virus in the National collection was GVA (60.0%), followed by GLRaV-1 (29.5%), GFkV (24.2%), GFLV (17.9%), ArMV (12.6%), GLRaV-2 and GVB (2.1%). In the Risika collection, GLRaV-3 was followed by GFLV (42.1%), GFkV (36.8%), GVA (32.6%), ArMV (23.2%), GLRaV-1 (11.6%), GLRaV-2 and GVB (1.1%). Mixed infections with two, three of four different viruses were also common in both collections. In the National collection the most common mixed infections were GLRaV-3 + GVA (15.8%) and GLRaV-1 + GLRaV-3 + GVA (14.7%), while in the Risika collection dominant were mixed infections with GLRaV-3 + GVA (10.5%) and GFLV + GLRaV-3 (8.4%). Free of all eight tested viruses were ten vines (10.5%) in the National collection and only seven vines (7.4%) in the Risika collection. This investigation has demonstrated that there has been deteriorated sanitary status of Croatian autochthonous cultivars, and indicates the need for the production of certified virus-free planting material.

Key words: native cultivars, ELISA, grapevine viruses.

Introduction

Viticulture is a vital part of the Croatian national economy with about 15% of the country's inhabitants directly or indirectly involved in viticulture. At the end of the 19th and the beginning of the 20th century Croatia had about 200,000 hectares of vineyards, but presently there are only 25,000 ha. The general division of the Croatian vine-growing regions is in two distinct climatic regions - the "Continental" region with features of a continental - type climate (middle European), and the "Coastal" region with a pronounced influence of the Adriatic Sea (Mediterranean-type climate). In the beginning of 20th century more then 400 cultivars were grown in Croatia (Jelaska and Briza, 1967), while in the mid 20th century many of the native cultivars had been lost because of vineyard destruction caused by new fungal diseases (e.g. *Plasmopara viticola*, *Uncinula necator*) and pests (mainly *Phylloxera vastatrix*) which caused rapid abandoning of wine production. The demands of

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modern production and the introduction of world-known cultivars (e.g. Chardonnay, Riesling, Cabernet Sauvignon, Merlot) also reduced grapevine biodiversity. In the last decade, many efforts in preservation of grapevine biodiversity in Croatia have been undertaken. Until now detailed inspection of the Croatian vine-growing regions has been done in order to locate all remaining native cultivars, paying special attention to the Dalmatian region, but not disregarding the others (the North Adriatic region and the Continental region).

Investigations have shown that it is still possible to find large numbers of native cultivars, much more than expected when initiating the quest. However, many of these cultivars have no economic importance, and some of them are endangered with only a few stocks left. Each identified native cultivar has been assigned a unique number, and they have been planted in the National collection of Croatian native grape varieties located at the Jazbina experimental station at the Faculty of Agriculture in Zagreb. Each genotype in the collection is represented with six vines, and to date the collection contains more than 120 different cultivars. As well, few regional collections were established with the aim to save duplicates and also to enable their evaluation for grape production in appropriate climatic conditions. One of these collections was established during 2004 at Risika on the island Krk. This collection emerged as a result of a grapevine inventory collection in the North Adriatic region and it contains 19 cultivars which are considered to be native for that region. Each cultivar is represented by at least 25 plants and their genetic, ampelographic and economic evaluations are in progress. In most cases plants in this collection are produced using budwood from different mother plants found in different vineyards of the North Adriatic region. Budwood from single mother plants was used only in cases of cultivars with very narrow production region or those where only single mother plants were found.

Both collections have been established using certified rootstocks, SO4 in the National collection and Richter 110 in the Risika collection. Most of the cultivars were grafted using chip-budding on previously planted rootstocks, and some of the vines were grafted using the green grafting technique. Soil in both collections was not tested for the presence of nematodes before planting, but both locations have histories of at least 10 years without vineyards.

The main purpose of the established collections is to preserve the Croatian native cultivars from extinction and to investigate their ampelographic characteristics. At the end of evaluation for those cultivars with unique genotype and good agronomic traits the collections will serve as base material for further multiplication. Results of this research will also be used for planning further activities regarding preservation and revitalization of native grapevine cultivars, with special accent to their sanitation.

Materials and methods

Plant material and symptoms observation

The surveys were conducted in the National collection located at the experimentation station Jazbina and the Risika collection located on the island Krk (Figure 1). During the 2008 vegetation period (before ELISA testing) and in 2010 (after ELISA results had been obtained) plants were visually inspected for symptoms that could be connected with virus infections. From each collection, 95 samples were taken during February 2009. Collected samples taken from the National collection belonged to 95 different cultivars while those taken from the Risika collection belonged to 19 different native cultivars. In order to obtain as much confidential results as possible, and to minimize false negative results due to uneven distribution of viruses in plants, the sample collected from each investigated plant comprised of at least three well wooded cuttings ca. 10 cm in length taken from different basal parts of each vine. The samples were labeled, placed in plastic bags and stored at 4°C before testing, which was completed within one month after collection.

Serological tests

All samples were tested for presence of eight viruses: *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Grapevine fleck virus* (GFkV), *Grapevine leafroll-associated virus 1* (GLRaV-1), *Grapevine leafroll-associated virus 2* (GLRaV-2), *Grapevine leafroll-associated virus 3* (GLRaV-3), *Grapevine virus A* (GVA) and *Grapevine virus B* (GVB). The viruses were detected using different

Figure 1. Locations of the National collection of Croatian native grape varieties at experimentation station Jazbina and the regional Risika collection on the island of Krk. Grey areas are vine-growing regions of the North Adriatic region from which native cultivars were collected and planted in the Risika collection.

types of ELISA: double antibody sandwich ELISA (DAS-ELISA) for ArMV, GFLV, GLRaV-1, GLRaV-2 and GLRaV-3; double antibody sandwich indirect ELISA (DASI-ELISA) for GFkV; protein A double antibody sandwich ELISA (protein A-DAS-ELISA) for GVA and antigen direct binding double antibody sandwich indirect ELISA for GVB. All reagents except substrate (p-nitrophenylphosphate, Sigma, USA) were provided by Agritest (Valenzano, Italy). From each cutting in sample cortical shavings were taken and mixed in average sample of 0.2 g, pulverized in mortar using pestle and liquid nitrogen to obtain better homogenization and extraction. Each sample was then diluted with 3 mL of grapevine extraction buffer. All other ELISA steps were conducted according to producer's instructions. After adding the substrate, plates were incubated for 2 h and absorbance values were measured on the BIOTEK EL800 spectrophotometer (BioTek, USA) at the wavelength of 405 nm. The samples with absorbance greater than three times the average value of negative controls were considered as positive.

Results

Symptoms of virus infections in both collections were detected from the beginning of vegetative growth as low vigor of plants, shoots with short internodes, flowering and pollination problems. The main symptoms which could be assigned to viruses from the leafroll complex were detected from the verasion stage and increased to the end of vegetative growth. They were expressed in form of downwards leaf rolling, premature reddening

of the leaves in black-berried cultivars and irregular fruit ripening. Since some of the vines in both collections were produced using chip-budding or green grafting techniques on previously planted rootstocks, problems with grafting success were also very common.

The results of the ELISA tests revealed that in both collections GLRaV-3 was the dominant virus with very similar levels of incidence – 75 samples (78.9%) in the National collection and 73 samples (76.8%) in the Risika collection. The second most frequent virus in the National collection was GVA occurring in 57 samples (60.0%), followed by GLRaV-1 – 28 samples (29.5%), GFkV – 23 samples (24.2%), GFLV – 17 samples (17.9%) and ArMV – 12 samples (12.6%). The other two viruses included in the investigation – GLRaV-2 and GVB - were found in only two (2.1%) of the 95 tested vines.

The situation in the Risika collection was slightly different, with GFLV as a second most frequent virus detected in 40 samples (42.1%), followed by GFkV – 35 samples (36.8%), GVA – 31 samples (32.6%), ArMV – 22 samples (23.2%) and GLRaV-1 – 11 samples (11.6%). Also in this collection, GLRaV-2 and GVB were the scarcest viruses, both found in only one sample (1.1%).

Multiple infections with two, three of even four viruses were also common in both collections (Figure 2). In the National collection the most frequent mixed infection combinations were GLRaV-3 + GVA (15.8%) and GLRaV-1 + GLRaV-3 + GVA (14.7%). In the Risika collection, the dominant mixed infection was GLRaV-3 + GVA (10.5%) followed by GFLV + GLRaV-3 (8.4%), while the most common mixed infections with three viruses were GFLV + GFkV + GLRaV-3 and GFkV + GLRaV-3 + GVA, both with incidence of 7.4%. Other multiple infections in both collections were less common, with incidences of less than 6%. To date, lethal effects in both collections caused by detected viruses or virus combinations was not observed. Only ten vines (10.5%) from the National collection, and seven vines (7.4%) from the Risika collection, were free of all tested viruses. Detailed sanitary status

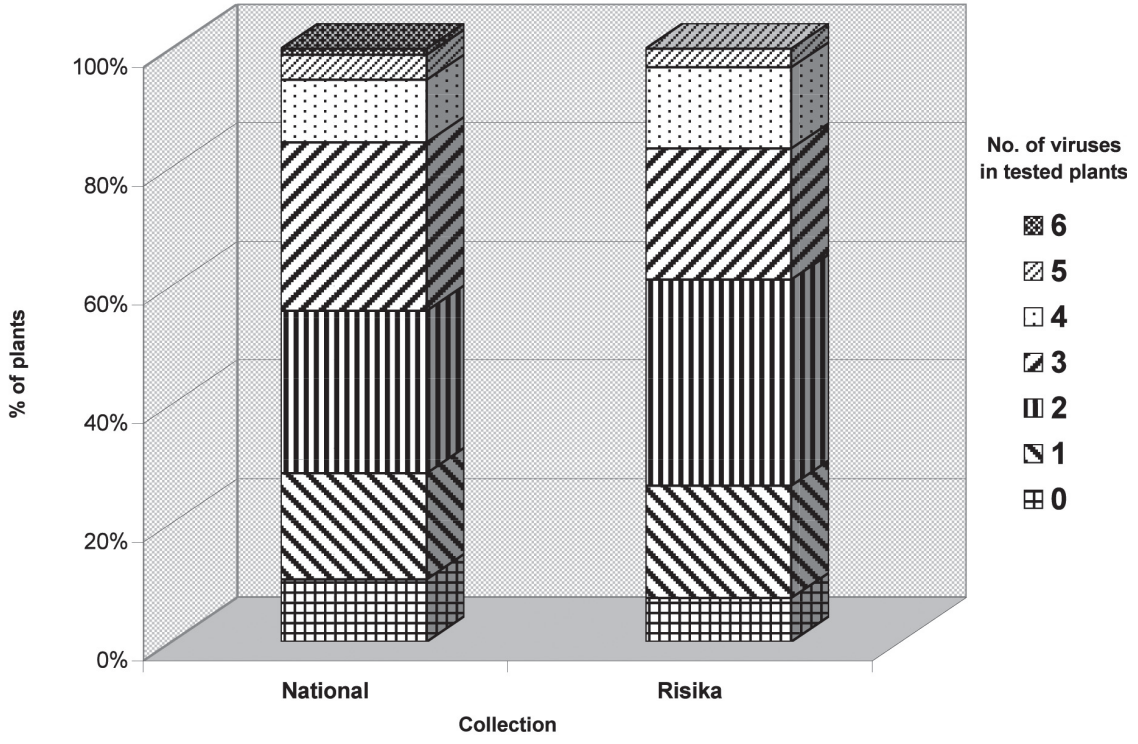

Figure 2. Percent ratio of plants from the National collection and from the Risika regional collection free of all eight tested viruses and those with single or multiple infections, as shown from ELISA tests.

Table 1. Incidence of virus infections, determined by ELISA tests, in the National collection of Croatian native grape varieties. ArMV - *Arabis mosaic virus*, GFLV - *Grapevine fanleaf virus*, GFkV - *Grapevine fleck virus*, GLRaV-1 - *Grapevine leafroll-associated virus 1*, GLRaV-2 - *Grapevine leafroll-associated virus 2*, GLRaV-3 - *Grapevine leafroll-associated virus 3*, GVA - *Grapevine virus A* and GVB - *Grapevine virus B*. Cultivars in bold were free of all eight tested viruses.

Cultivar	ArMV	GFLV	GFkV	GLRaV-1	GLRaV-2	GLRaV-3	GVA	GVB
Babica crna	+	+	+	0	0	+	+	0
Babić	0	+	+	0	0	+	+	0
Beli debejan	0	+	0	0	0	+	0	0
Bilan bijeli	+	+	+	0	+	+	0	0
Bljuzgavac	0	0	0	+	0	+	0	0
Bogdanuša	+	0	0	0	0	+	0	0
Bratkovina bijela	0	0	0	+	0	+	0	0
Bratkovina crvena	0	0	0	0	0	0	0	0
Cetinka	0	0	0	0	0	+	+	0
Cibib	0	0	0	0	0	+	+	0
Cipar	0	0	0	+	0	+	+	0
Crljenak kaštelanski	0	0	0	0	0	+	0	0
Crljenak viški	0	0	0	+	0	+	+	0
Crnka	0	+	0	+	0	+	+	0
Debejan	0	+	0	0	0	+	0	0
Debit	0	0	+	0	0	+	0	0
Dišeća ranina	0	0	+	+	0	+	+	0
Dobričić	0	+	0	+	0	+	+	0
Dolčin	+	0	0	0	0	0	0	0
Drnekuša mala	0	0	+	0	0	+	+	0
Drnekuša vela	0	0	0	0	0	+	+	0
Dugovrst bijeli	+	0	0	0	0	+	+	0
Fermentu	0	0	0	0	0	+	0	0
Galac crni	0	+	+	0	+	+	+	0
Garganja	0	0	0	0	0	+	0	0
Gegić	+	0	0	+	0	0	+	0
Glavanjuša	0	0	0	0	0	+	+	0
Glavinuša	0	0	0	+	0	+	+	0
Grk	0	0	0	0	0	+	0	0
Gustopupica	0	0	0	0	0	+	+	0
Jarbola	0	0	0	0	0	0	0	0
Kadarun	0	0	0	+	0	+	+	0
Katarina rikiki	0	0	0	0	0	+	+	0
Kraljevina	0	0	0	0	0	0	0	0
Krivaja crna	0	0	+	0	0	0	+	0
Krkošija	0	+	0	0	0	+	+	0
Krstićevica	0	0	0	0	0	+	+	0
Kuč	0	0	0	+	0	+	+	0
Kujundžuša	0	+	0	0	0	0	0	0
Kurtelaška	0	0	0	0	0	0	0	0
Lasina	0	0	0	+	0	+	+	0
Lelekuša	0	0	0	0	0	+	+	0
Lipovina	0	0	0	0	0	0	0	0
Ljutun crni	0	0	0	+	0	+	+	0
Malvasija dubrovačka	+	+	0	+	0	+	+	+
Malvasija istarska	0	0	+	0	0	+	+	0
Maraština	+	0	+	0	0	+	0	0
Mekuja	0	0	0	+	0	+	+	0
Mijajuša	0	0	0	+	0	+	+	0
Mladenka	0	0	0	0	0	+	+	0
Moslavac	0	0	0	0	0	0	0	0
Muškat ruža omiški	0	0	0	+	0	+	+	0
Muškatel	0	0	0	0	0	+	+	0

continues

Table 1. *continued*

Cultivar	ArMV	GFLV	GFkV	GLRaV-1	GLRaV-2	GLRaV-3	GVA	GVB
Ninčuša	0[a]	0	0	0	0	+	0	0
Palagružanka bijela	0	0	0	0	0	+	0	0
Palaruša viška	0	0	+	0	0	+	+	0
Pavicić	+[a]	+	0	0	0	+	+	0
Petovka	0	0	+	0	0	+	+	0
Plavac mali crni	0	0	0	0	0	0	+	0
Plavac mali sivi	0	+	0	0	0	+	+	0
Plavec žuti	0	0	0	0	0	+	0	0
Plavina	0	0	0	0	0	0	0	0
Pošip bijeli	0	0	0	+	0	+	0	0
Pošip crni	0	0	+	+	0	+	+	0
Pošipica bijela	0	0	0	+	0	+	+	0
Prč bijdeli	0	0	0	0	0	+	+	0
Pršljivka	0	0	0	0	0	0	0	0
Ranfol	0	0	0	+	0	0	0	0
Razaklija	0	0	+	0	0	+	0	0
Rogoznička	0	0	0	+	0	+	+	0
Rudežuša	+	+	+	0	0	+	0	0
Ruža bijela	0	0	0	0	0	+	+	0
Silbijanac	0	0	+	0	0	+	+	0
Siložder crni	0	0	0	+	0	+	+	+
Stara brajda	0	0	0	0	0	+	+	0
Stara braničevka	0	0	0	0	0	0	0	0
Susac	0	0	0	0	0	+	+	0
Švrdlovina crna	0	0	+	+	0	+	+	0
Šarica trišnjavica	0	+	+	0	0	+	+	0
Šemperinka crna	0	0	0	0	0	+	0	0
Šipelj	0	0	0	0	0	0	0	0
Škrlet	+	0	0	0	0	0	0	0
Tanetova loza	0	0	0	0	0	+	0	0
Teran	0	0	0	0	0	+	0	0
Topol	+	+	+	0	0	+	+	0
Trojišćina	0	+	0	0	0	+	+	0
Vlaška bijela	0	0	0	+	0	+	+	0
Vranac	0	0	+	0	0	0	0	0
Vrbić	0	0	0	0	0	+	+	0
Zadarka	0	0	+	+	0	0	+	0
Zelenjak crni	0	0	0	+	0	+	+	0
Zlatarica vrgorska	0	0	0	0	0	+	0	0
Žilavka	0	0	0	0	0	+	+	0
Žlahtina	0	0	+	0	0	+	+	0
Žumić	0	0	+	+	0	0	0	0
TOTAL (95)	12	17	23	28	2	75	57	2
Percent	12.6	17.9	24.2	29.5	2.1	78.9	60.0	2.1

[a] +, positive, virus detected; 0 negative, virus not detected.

of all plants included in the surveys, in terms of virus infections, is outlined in Tables 1 and 2.

Discussion

The results of the surveys conducted in the collections of Croatian native grapevine cultivars revealed presence of important viruses in different levels of incidence extends and high rates of their infections. Since vines in the collections have been widely gathered from vine-growing regions, although large number of plants were not analyzed, our results give a good estimation of the Croatian sanitary status of autochthonous cultivars. Our results are in agreement with those from previous studies made on larger numbers of samples (Poljuha *et al*., 2004; Voncina *et al.,* 2008; Karoglan Kontic *et al*., 2009; Voncina *et al,*. 2009a; Voncina *et*

Table 2. Incidence of virus infections determined by ELISA, test in the regional Risika collection of Croatian native grape varieties. ArMV - *Arabis mosaic virus*, GFLV - *Grapevine fanleaf virus*, GFkV - *Grapevine fleck virus*, GLRaV-1 - *Grapevine leafroll-associated virus 1*, GLRaV-2 - *Grapevine leafroll-associated virus 2*, GLRaV-3 - *Grapevine leafroll-associated virus 3*, GVA - *Grapevine virus A* and GVB - *Grapevine virus B*. Cultivars in bold were free of all eight tested viruses.

Cultivar	ArMV	GFLV	GFkV	GLRaV-1	GLRaV-2	GLRaV-3	GVA	GVB
Bašćan v[a]. 2	0	0	+	0	0	+	+	0
Bašćan v. 3	0	0	+	0	0	+	+	0
Bašćan v. 4	0	0	+	0	0	+	+	0
Bašćan v. 5	+	0	+	0	0	+	+	0
Bašćan v. 6	+	0	+	0	0	+	0	0
Beli debejan v. 1	0	+	0	0	0	+	0	0
Beli debejan v. 2	0	+	0	0	0	+	0	0
Beli debejan v. 3	0	+	+	+	0	+	0	+
Beli debejan v. 4	0	+	0	0	+	+	+	0
Beli debejan v. 6	0	+	0	0	0	+	+	0
Bilan bijeli v. 4	0	+	+	0	0	+	0	0
Bilan bijeli v. 5	+	+	+	0	0	+	0	0
Bilan bijeli v. 6	0	+	+	+	0	+	0	0
Bilan bijeli v. 7	0	0	+	0	0	+	0	0
Bilan bijeli v. 8	0	+	+	0	0	0	+	0
Brajdica bijela v. 1	0	+	+	0	0	+	0	0
Brajdica bijela v. 2	0	+	+	0	0	+	0	0
Brajdica bijela v. 3	0	+	+	0	0	+	0	0
Brajdica bijela v. 4	0	+	+	0	0	+	0	0
Brajdica bijela v. 5	0	+	+	0	0	+	0	0
Debejan v. 1	0	+	0	0	0	+	0	0
Debejan v. 2	0	0	+	0	0	+	+	0
Debejan v. 3	+	0	0	+	0	+	+	0
Debejan v. 4	0	0	+	0	0	+	0	0
Debejan v. 5	0	0	0	0	0	+	+	0
Jarbola v. 1	0	0	0	0	0	0	0	0
Jarbola v. 2	0	0	0	0	0	0	0	0
Jarbola v. 3	0	0	+	0	0	0	0	0
Jarbola v. 4	+	0	0	0	0	0	0	0
Jarbola v. 5	0	0	0	0	0	0	0	0
Kamenina v. 1	+	0	0	+	0	+	0	0
Kamenina v. 3	+	0	0	0	0	0	0	0
Kamenina v. 4	0	+	0	0	0	0	0	0
Kamenina v. 5	0	+	0	0	0	0	0	0
Kamenina v. 6	0	0	0	0	0	+	0	0
Katarina rikiki v. 1	0	0	0	0	0	+	+	0
Katarina rikiki v. 2	0	0	0	0	0	0	0	0
Katarina rikiki v. 3	0	0	0	0	0	+	0	0
Katarina rikiki v. 4	0	0	0	0	0	+	+	0
Katarina rikiki v. 5	0	0	0	0	0	+	+	0
Ošljevina v. 2	+	+	+	0	0	+	0	0
Ošljevina v. 3	0	0	+	0	0	+	0	0
Ošljevina v. 4	0	+	+	0	0	+	0	0
Ošljevina v. 5	0	0	+	0	0	+	0	0
Ošljevina v. 6	+	+	+	0	0	+	0	0
Plavac v. 1	0	+	0	0	0	+	0	0
Plavac v. 2	+	+	0	+	0	+	0	0
Plavac v. 3	0	+	0	0	0	+	0	0
Plavac v. 4	0	+	0	+	0	+	0	0
Plavac v. 5	+	+	0	0	0	+	0	0
Plavčina v. 2	0	+	+	0	0	+	+	0
Plavčina v. 4	0	+	+	0	0	+	+	0
Plavčina v. 6	+	+	+	0	0	+	+	0
Plavčina v. 8	0	0	+	0	0	+	+	0
Plavčina v. 10	0	0	+	0	0	+	+	0
Rožeta v. 1	0	0	0	0	0	+	0	0

continues

Table 2. *continued*

Cultivar	ArMV	GFLV	GFkV	GLRaV-1	GLRaV-2	GLRaV-3	GVA	GVB
Rožeta v. 6	0	0	0	0	0	+	0	0
Rožeta v. 7	0	0	0	0	0	+	0	0
Rožeta v. 9	0	0	0	0	0	+	0	0
Rožeta v. 10	+	0	0	0	0	+	0	0
Rušljin v. 1	0	+	+	0	0	0	0	0
Rušljin v. 2	0	+	+	0	0	0	0	0
Rušljin v. 3	0	+	+	0	0	0	0	0
Rušljin v. 4	0	+	+	0	0	0	0	0
Rušljin v. 5	0	+	+	0	0	0	0	0
Sansigot v. 1	0	0	0	0	0	0	0	0
Sansigot v. 2	0	0	0	0	0	0	0	0
Sansigot v. 3	+	0	0	0	0	0	0	0
Sansigot v. 4	0	0	0	0	0	0	+	0
Sansigot v. 6	+	0	0	0	0	+	0	0
Sušić v. 1	0	+	0	0	0	+	0	0
Sušić v. 2	+	+	0	+	0	+	0	0
Sušić v. 3	0	+	0	0	0	+	0	0
Sušić v. 4	+	+	0	0	0	+	0	0
Sušić v. 5	0	+	0	0	0	+	0	0
Trojišćina v. 1	0	+	0	0	0	+	+	0
Trojišćina v. 2	0	0	0	0	0	+	+	0
Trojišćina v. 3	+	0	0	+	0	+	+	0
Trojišćina v. 4	0	0	0	0	0	+	+	0
Trojišćina v. 5	0	0	0	0	0	+	+	0
Volarovo v. 1	0	0	0	0	0	+	0	0
Volarovo v. 2	+	0	0	0	0	+	0	0
Volarovo v. 3	0	0	0	0	0	+	0	0
Volarovo v. 4	0	0	0	0	0	+	0	0
Volarovo v. 5	0	0	0	0	0	+	0	0
Vrbić v. 1	0	0	0	0	0	+	0	0
Vrbić v. 2	0	0	0	0	0	0	0	0
Vrbić v. 3	0	0	0	0	0	+	+	0
Vrbić v. 4	+	+	0	+	0	+	+	0
Vrbić v. 5	+	0	0	0	0	0	+	0
Žumić v. 1	0	0	0	+	0	+	0	0
Žumić v. 2	0	0	0	0	0	+	+	0
Žumić v. 3	+	0	0	+	0	+	+	0
Žumić v. 4	0	0	+	0	0	0	+	0
Žumić v. 5	0	0	0	0	0	+	+	0
TOTAL (95)	22	40	35	11	1	73	31	1
Percent	23.2	42.1	36.8	11.6	1.1	76.8	32.6	1.1

+ positive, virus detected; 0 negative, virus not detected, v[a] - vine

al. 2009b). In all these studies, GLRaV-3 was the dominant virus, with incidence rates varying from 72 to 100%.

The reason of such dominance of GLRaV-3, especially in the Coastal region of Croatia, remains unknown. A possible explanation for spread at a site could be in presence of the insect vestors mealybug (*Planococcus ficus*) and soft scales (*Pulvinaria vitis*, *Neopulvinaria innumerabilis*) since these insects are very common in Coastal region (Masten Milek, 2009). Another potential explanation, especially for rare cultivars with very narrow numbers of potential mother plants, is that GLRaV-3 is disseminated through budwood from infected mother plants. The virus status was also similar to those determined in other countries of Mediterranean region. Beside Croatia, GLRaV-3 is the dominant virus in Mediterranean region, including most vine-growing regions of Italy (Savino *et al*. 2001), and also in Algeria, Cyprus, Greece, Malta, Morocco and Tunisia (Digiaro *et al*. 2000).

Comparitive analysis of the results from both collections revealed similar frequency of GLRaV-3, GLRaV-2 and GVB in both the National collection and Risika regional collection. Frequency of the other viruses varied significantly between the two collections, especially in case of GVA, with 27.4% difference in occurrence of this virus between the National and the regional collection. As previously reported (Voncina *et al.* 2010, Voncina *et al.* 2011), GLRaV-2 and GVB are not commonly detected viruses within the populations of Croatian native grapevine germplasm. Since all plants in both collections were grafted on certified rootstocks, entrance of viruses through infected rootstocks is likely to be reduced to minimum. The same sanitary status of some cultivars in the Risika collection is likely to have resulted from their multiplication using budwood from the same mother plants (Bašćan vines 2, 3, 4; Beli debejan 1 and 2; Bilan 4 and 6; Brajdica bijela; Kamenina 4 and 5; Katarina 1, 4 and 5; Ošljevina 3 and 5; Plavac 1, 3 and 4; Plavčina 2 and 4; Rožeta 1, 6, 7, 9; Rušljin; Sušić 1, 3 and 5; Trojišćina 1, 4 and 5; Volarovo 1, 3, 4 and 5). This clearly demonstrates that long distance dissemination of viruses occurs as a consequence of using budwood from infected mother plants. The fact that some of the budwood was collected from isolated areas where only rare, native, self-rooted cultivars are grown without foreign cultivars in close vicinity, supports the conclusion that a wide range of grapevine viruses has been present in Croatia for a long time.

Since the aim of the National collection is to include all native cultivars, buds from vines with confirmed native status from the Risika collection were used for production of planting material for the National collection. In this case, from the sanitary status of some cultivars we can clearly see from which plant in the Risika collection the budwood was taken – Beli debejan in the National collection is raised using budwood from Risika's Beli debejan vine 2; Debejan using buds from Debejan vine 1, Trojišćina from Trojišćina vine 1 and Vrbić from Vrbić vine 3. This also demonstrates that for most grapevine viruses or at least those considered economically important, infected planting material (budwood, rootstocks, grafted vines) represents their main mode of dissemination. Furthermore, from the sanitary status of the cultivars Brajdica bijela and Rušljin planted in the Risika collection, it is evident that all their vines were produced using budwood from single mother plants.

The currently valid Croatian legislation for grapevine planting material requires that in nurseries incidence of four viruses (ArMV, GFLV, GLRaV-1 and GLRaV-3), must be reduced to minimum rates, while rootstocks must additionally be tested for the presence of *Grapevine fleck virus* (GFkV). Beside legislation amendments, viticulture in Croatia is currently faced with several obstacles. Firstly, there is no certified planting material of native cultivars on the market. Secondly, obtained results (especially from the Risika collection where unique and very rare cultivars are grown) revealed that in case of cultivars with very small areas of production virus-free mother plants are practically impossible to find. Thirdly, the poor sanitary status of available native planting material means that further dissemination of viruses is very likely. Fourthly, the high incidence of virus infections of almost all Croatian native cultivars makes their real genetic potential and agronomic traits still unknown. All of these factors may contribute to the abandoning of some native cultivars, genetic erosion and increased risk of loosing some genotypes. The negative impacts of different viruses on different foreign cultivars are well known (Martelli *et al*., 1986; Walter and Martelli, 1996; Bertamini *et al*., 2004; Besse *et al.*, 2009; Golino *et al.*, 2009; Legorburu *et al*., 2009). At the same time information about negative impacts of viruses on Croatian native cultivars are very scanty or unknown. Recently, only a survey assessing the impact of GLRaV-3 on cultivar Grk was conducted (Preiner *et al*., 2010). Results showed differences in some philometric parameters, mechanical structure of grapes and pH value of the must between infected and healthy plants. One significant result was the recording of a higher percent of normal size berries (pollinated flowers) in GLRaV-3 infected vines.

Some of the Croatian native cultivars are currently not showing good potential for production because of poor agronomic traits (low sugar content, increased acids). According to the results of their sanitary status (especially high infection rate with GLRaV-3), their limited performance partially due to poor sanitary status cannot be excluded. Once these cultivars are made virus-free (through thermotherapy and meristem- or shoot-

tip culture) they may give better performance and become valuable for nurseries and vine producers. First experience with virus-free native cultivars from recently established collection fields have given very promising results. Significant differences in vigor, growth and yield between infected and virus-free plants of the same cultivars were recorded (Maletic and Preiner, personal communication). Such examples with foreign cultivars are not rare, clearly demonstrating that elimination of some viruses can significantly improve agronomic performance (Guidoni *et al*., 1997; Mannini and Credi, 2000; Mannini *et al*., 2009) and have positive impacts on grape industry (La Notte *et al*., 2009).

In conclusion, in this survey we provide clear evidence that appropriate sanitation procedures will be necessary in the future for many Croatian native grapevine cultivars in order to provide healthy mother plants for production of certified virus-free planting material. This will also allow comparison field studies carried out to reveal the negative impacts of different viruses on Croatian native grapevine cultivars in the appropriate climatic regions. Since the process from sanitation to production of certified planting material takes considerable time, mother plants which winegrowers use as budwood sources, should be tested for at least the economically important viruses. If they are infected, particularly with multiple virus infections or with viruses disseminated by infected planting material, they should be excluded as propagation sources.

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