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Distribution of nine viruses in Croatian autochthonous grapevine (*Vitis vinifera* L.) cultivars from Dalmatian region included in clonal selection

Distribucija devet virusa kod hrvatskih autohtonih sorata vinove loze (*Vitis vinifera* L.) s područja Dalmacije uključenih u klonsku selekciju

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Abstract

The investigation of virus incidence was conducted on 14 autochthonous Croatian grapevine cultivars on vines from 51 vineyards located in the Dalmatian region. A total of 1,116 vines were tested by ELISA (enzyme-linked immunosorbent assay) on nine viruses: *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Grapevine fleck virus* (GFkV), *Grapevine leafroll-associated virus 1*, *2*, *3* and *7* (GLRaV-1, GLRaV-2, GLRaV-3 and GLRaV-7), *Grapevine virus A* and *B* (GVA and GVB). ELISA confirmed presence of eight viruses: GLRaV-3 in 888 vines (79.6%), GVA 685 (61.4%), GLRaV-1 455 (40.8%), GFkV 223 (19.9%), GFLV 219 (19.6%), GLRaV-2 46 (4.1%), ArMV 36 (3.2%) and GVB 35 (3.1%). Altogether 93 vines (8.3%) were free of all tested viruses, 168 (15.1%) were infected with a single virus, 368 (32.9%) with two, 314 (28.1%) with three, 127 (11.4%) with four, 43 (3.9%) with five, and 3 (0.3%) vines with six viruses. Symptoms of premature leaf color change and downward curling of leaf blades were common, while abnormal branching, short internodes, double nodes and irregular ripening were detected sporadically. The investigation confirms deteriorated sanitary

status of all cultivars except Grk and Plavina and indicates the necessity of intensifying grapevine clonal and sanitary selection programs.

Keywords: autochthonous grapevine cultivars, ELISA, grapevine viruses, symptoms

Sažetak

Istraživanje učestalosti pojave virusa provedeno je kod 14 hrvatskih autohtonih sorata vinove loze na trsovima iz 51 vinograda na području Dalmacije. Ukupno 1116 trsova je analizirano metodom ELISA (enzyme-linked immunosorbent assay) na devet virusa: virus mozaika gušarke (ArMV), virus lepezastog lista vinove loze (GFLV), virus pjegavosti vinove loze (GFkV), uvijenosti lista vinove loze pridružene viruse 1, 2, 3 i 7 (GLRaV-1, GLRaV-2, GLRaV-3 i GLRaV-7), A- i B-virus vinove loze (GVA i GVB). Metodom ELISA potvrđena je prisutnost osam virusa: GLRaV-3 u 888 trsova (79.6%), GVA 685 (61.4%), GLRaV-1 455 (40.8%), GFkV 223 (19.9%), GFLV 219 (19.6%), GLRaV-2 46 (4.1%), ArMV 36 (3.2%) i GVB 35 (3.1%). Sveukupno kod 93 trsa (8.3%) nije detektirana prisutnost istraživanih virusa, kod 168 (15.1%) je utvrđena zaraza samo jednim, 368 (32.9%) sa dva, 314 (28.1%) sa tri, 127 (11.4%) sa četiri, 43 (3.9%) sa pet i kod 3 (0.3%) trsa istovremena zaraza sa šest virusa. Simptomi prijevremene promjene boje lišća i uvijanja rubova plojke prema naličju su bili dosta česti, dok je nepravilno grananje, skraćeni internodiji, duplih nodiji i neujednačeno dozrijevanje zabilježeno sporadično. Istraživanje je potvrdilo loše zdravstveno stanje u pogledu zaraze virusima kod svih sorata osim kod Grka i Plavine, i ukazalo na nužnost intenziviranja programa klonske i zdravstvene selekcije.

Ključne riječi: autohtone sorte vinove loze, ELISA, simptomi, virusi vinove loze

Introduction

Grapevines in Croatia represent one of the most common woody plants, especially in Coastal region where Greek colonists brought the winegrowing tradition. According to Jelaska and Briza (1967), at the beginning of the 20th century, 400 different grapevine cultivars (*Vitis vinifera* L.) were grown in Croatia. However, approximately fifty years later, many autochthonous cultivars were irreversibly lost due to phylloxera (*Viteus vitifoliae* Fitch, sin. *Phylloxera vastatrix* Plan) and fungal diseases (downey mildew - *Plasmopara viticola* Berk. & M.A. Curtis and powdery mildew - *Erysiphe necator* Schwein.). This resulted in the usage of non-certified planting material for new vineyards and introduction of foreign cultivars and American rootstocks. Although this solved the phylloxera problem, new situation created the opportunity for viral diseases, present throughout the world, to be introduced to Croatia. According to Maletić et al. (2015) Croatia is characterized by at least 125 autochthonous grapevine cultivars. In 2015 there were 20,392 ha of vineyards: 10,590 ha in Continental region and 9,802 ha in Coastal region (Croatian Bureau of Statistics, 2015). While in the Continental region of the country autochthonous cultivars have a symbolic role, in Coastal region they have important role and are grown on significant

agricultural surfaces. The most important and widespread red berry cultivar in Dalmatia is Plavac mali, followed by Plavina and Babić, while the most widespread white berry cultivars are Maraština, Pošip and Vugava. Although viticulture in Croatia has a long tradition and represents a very important branch of agriculture, comprehensive work on clonal selection and evaluation of the sanitary status of autochthonous, as well as introduced grapevine cultivars, started just 20 years ago. As a result, there are currently only nine autochthonous cultivars with certified planting material (free from *Arabis mosaic virus* - ArMV, *Grapevine fanleaf virus* - GFLV, *Grapevine leafroll-associated virus 1* and *3* - GLRaV-1 and GLRaV-3) available on the market. Otherwise, most of Croatia's autochthonous grapevine cultivars planting material has poor, or at least questionable sanitary status, and there are no certified primary sources. Today, seven of the most important autochthonous cultivars are included in the small-scale program of clonal and sanitary selection (Plavac mali, Plavina, Pošip, Vugava, Grk, Debit and Maraština). However, this is still insufficient because of the increasing demand for planting material and no certified planting material for more than 50 other autochthonous cultivars used for wine production.

This survey was conducted in response to the lack of information, at the time of the survey, regarding the distribution of *Grapevine fleck virus* (GFkV), Grape*vine leafroll-associated virus 7* (GLRaV-7) and *Grapevine virus A* (GVA) in Dalmatia, but also in Croatia in general. Since deteriorated sanitary status of autochthonous cultivars may be one of the major reasons for lack of interest, this survey was made on vines included in clonal selection with the aim of finding virus-free vines, especially of endangered cultivars, that can serve as potential mother plants for the production of certified planting material.

Materials and methods

Screening and collecting of samples

The samples were collected from 51 commercial vineyards located in Dalmatia from vines included in mass positive clonal selection. Evaluation of their agronomic traits (yield, cluster and berry characteristics, sugar and acid ratio, tolerance to fungal diseases such as grey mold, powdery mildew, downy mildew, dead-arm etc.) together with visual inspection for virus symptoms were done over a three-year period. All changes on vines' leaves, shoots and wood that could potentially be caused by virus infections were recorded in order to compare with the results of laboratory tests (enzyme-linked immunosorbent assay - ELISA). During the autumn of 2008 samples of each cultivar were collected from their most important growing region(s): Primošten (cv. Babić); surroundings of Šibenik (cvs. Babić, Maraština, Plavina); region of Kaštela (cvs. Babica, Dobričić, Glavinuša, Ljutun, Mladenka, Ninčuša, Vlaška); island Korčula (cvs. Grk, Maraština, Plavac mali, Plavina, Pošip); island Hvar (cvs. Maraština, Plavac mali); island Vis (cvs. Vugava, Plavac mali); island Svetac (cvs. Maraština, Plavac mali); peninsula Pelješac (cv. Plavac mali) and surroundings of Čitluk (cv. Maraština). Each sample was represented by at least three well-wooded cuttings taken from different basal part. Samples were labeled, stored in a refrigerator at 4 °C, and tested within 60 days. The number of samples taken from each cultivar roughly mirrored their importance: Plavac mali, as the most

important red berry cultivar, had the greatest number of samples (284) taken from 20 different locations/vineyards. Other cultivars were collected as follows: Babica (90 samples/4 locations), Babić (98/4), Dobričić (3/1), Glavinuša (16/1), Grk (70/4), Ljutun (85/3), Maraština (88/6), Mladenka (60/2), Ninčuša (24/2), Plavina (20/2), Pošip (103/4), Vlaška (49/2) and Vugava (126/5).

Serological assays

Vines were tested using ELISA for the presence of nine viruses: ArMV, GFLV, GFkV, GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-7, GVA and *Grapevine virus B* (GVB). All reagents were provided by Agritest (Valenzano, Italy). Cortical shavings taken from well-wooded cuttings were used as a potential source of antigen, frozen using 6 ml of liquid nitrogen and pulverized in mortar using pestle. Pulverized samples were diluted in 3 ml of grapevine extraction buffer (ratio 1:15, w:v) and all other ELISA-steps were carried out according to the manufacturer's instruction. Two hours after adding p-nitrophenylphosphate (Sigma, USA), results were measured on an EL800 spectrophotometer (BioTek, USA) at a wavelength of 405 nm.

Results and discussion

Virus symptoms

Field surveys confirmed different expression levels of symptoms: some ELISA positives clearly correlated with symptoms observed in the field, but many positives did not exhibit notable symptoms. In many vines infected with GLRaV-3 symptoms of leaf rolling and premature color changes (reddening or yellowing) in interveinal leaf area were observed. These symptoms were more commonly found on red berry cultivars grown in Kaštela region, Plavac mali on two locations on island Hvar, and Pošip on two locations on island Korčula. Similar symptoms were also observed on most of the Grk vines from island Korčula in which the presence of viruses from leafroll complex were not confirmed by ELISA. It has been assumed that those symptoms were induced by phytoplasmas, especially because presence of "*Candidatus* Phytoplasma asteris" and *Bois noir* had been earlier confirmed in cv. Grk and some other autochthonous cultivars grown in Coastal region (Mikec et al., 2006).

Vines positive on viruses from infective degeneration group (ArMV and GFLV) generally exhibited mild or no symptoms on leaves, but those positive for GFLV showed symptoms on shoots and bunches. Changes like abnormal branching, double nodes, shortened internodes with uneven ripening of berries within bunches and poor berry setting were most evident on cv. Plavac mali from several locations, but also on some vines of cvs. Vugava and Pošip. In all surveyed locations almost all vines of cv. Babić exhibited evident swelling above the graft region. Since bark was not removed from vines infected with GVA, changes on wood were not recorded, but vines infected with GLRaV-3 and GVA had more severe leaf roll symptoms and more evident color changes than vines infected only with GLRaV-3.

Serological assays

The ELISA-results confirmed high infection rates in 12 out of 14 autochthonous cultivars. Out of 1,116 tested vines, 1,023 (91.67%) were infected with at least one virus. In eight cvs. (Babica, Babić, Dobričić, Glavinuša, Ljutun, Mladenka, Ninčuša and Vlaška) no plant free of tested viruses was found (Table 1). Similar results were reported by Grenan et al. (2000) for less important cultivars in France.

Table 1. Review of virus infection incidence determined by ELISA on 14 Croatian autochthonous grapevine cvs collected from 51 vineyards

Cultivar No. of locations	No. of tested vines	Infected vines No. %	No. of vines infected with certain virus (%)							
			ArMV	GFLV	GFkV	GLRaV-1	GLRaV-2	GLRaV-3	GVA	GVB
Babica 4	90	90 100	5 5.6	42 46.5	30 33.3	90 100	1 1.1	90 100	77 85.6	0
Babić 4	98	98 100	2 2	5 5.1	23 23.5	4 4.1	33 33.7	98 100	81 82.7	4 4.1
Dobričić 1	3	3 100	0	0	0	2 66.67	1 33.33	3 100	3 100	2 66.67
Glavinuša 1	16	16 100	0	2 18.8	0	15 93.8	2 12.5	16 100	16 100	0
Grk 4	70	33 47.1	1 1.4	13 18.6	3 4.3	0	0	19 27.1	5 7.1	0
Ljutun 4	85	85 100	10 11.8	25 29.4	4 4.7	83 97.7	0	85 100	68 80	0
Maraština 6	88	83 94.3	2 2.3	12 13.6	15 17.1	23 26.1	1 1.1	70 79.6	60 68.2	2 2.3
Mladenka 2	60	60 100	0	0	16 26.3	21 35	0	60 100	41 68.3	15 25
Ninčuša 2	24	24 100	0	3 12.5	0	20 83.3	0	24 100	20 83.3	1 4.2
Plavac mali 20	284	263 92.6	15 5.3	89 31.3	43 15.1	136 47.9	7 2.5	194 68.3	194 68.3	6 2.1
Plavina 2	20	9 45	0	0	3 15	2 10	0	5 25	2 10	0
Pošip 4	103	88 85.4	1 0.9	13 12.6	35 33.9	41 39.8	0	61 59.2	16 15.5	1 0.9
Vlaška 2	49	49 100	0	9 18.4	6 12.3	13 26.5	0	49 100	8 16.3	3 6.1
Vugava 5	126	122 96.8	0	6 4.8	45 35.7	5 3.9	1 0.8	114 90.5	94 74.6	1 0.8
Total %	1,116	1,023 91.7	36 3.2	219 19.6	223 19.9	455 40.8	46 4.1	888 79.6	685 61.4	35 3.1

Total number of sampling locations, number of analyzed vines and those infected with at least one virus is given together with number/percentage of vines infected with certain virus.

From the data shown in Table 2 only 93 vines (8.3%) were free from all nine viruses included in investigation. Only in cvs. Grk and Plavina relatively high percent of virus-free vines was found – 52.9% and 55%, respectively. Beside mentioned, virus-free vines were also found in cvs. Pošip (14.6%), Plavac mali (7.4%), Maraština (5.7%) and Vugava (3.2%). According to these results, and those from the other authors, it is very difficult to find virus-free vines in the Coastal region, but situation improves significantly towards the Continental region (Poljuha et al., 2004; Zdunić et al., 2008; Karoglan Kontić et al., 2009a, 2009b; Zdunić et al., 2009; Poljuha et al., 2010; Vončina et al., 2011a, 2011b, 2011c).

Table 2. Vines infected with different number of viruses determined by ELISA

Cultivar	No. of tested vines	No. of vines free from all nine tested viruses %	No. of vines infected with different no. of viruses %					
			1	2	3	4	5	6
Babica	90	0	0	6 6.7	33 36.7	33 36.7	16 17.8	2 2.2
Babić	98	0	6 6.1	47 47.9	32 32.7	11 11.2	2 2	0
Dobričić	3	0	0	1 33.3	0	1 33.3	1 33.3	0
Glavinuša	16	0	0	1 6.3	11 68.8	4 25	0	0
Grk	70	37 52.9	26 37.1	6 8.6	1 1.4	0	0	0
Ljutun	85	0	0	16 18.8	42 49.4	18 21.2	9 10.6	0
Maraština	88	5 5.7	18 20.5	33 37.5	27 30.7	5 5.7	0	0
Mladenka	60	0	4 6.7	26 43.3	24 40	5 8.3	1 1.7	0
Ninčuša	24	0	0	8 33.3	12 50	4 16.7	0	0
Plavac mali	284	21 7.4	31 10.9	106 37.3	75 26.4	39 13.7	12 4.2	0
Plavina	20	11 55	6 30	3 15	0	0	0	0
Pošip	103	15 14.6	35 33.9	34 33	15 14.6	1 0.9	2 1.9	1 0.9
Vlaška	49	0	23 46.9	16 32.6	7 14.3	3 6.1	0	0
Vugava	126	4 3.2	19 15.1	65 51.6	35 27.8	3 2.4	0	0
Total %	1,116	93 8.3	168 15.1	368 32.9	314 28.1	127 11.4	43 3.9	30.3

All vines were tested on the presence of nine viruses (ArMV, GFLV, GFkV, GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-7, GVA and GVB).

Besides single infected vines (15.1%), a significant number of those infected with different combinations of two (32.9%), three (28.2%) or four (11.4%) viruses was found. Out of 168 vines with single infection, GLRaV-3 was found in 95 vines (8.5%), GFkV in 22 (1.9%), GLRaV-1 in 21 (1.9%), GFLV in 19 (1.7%) and GVA in 11 (0.9%) vines. A similar study conducted on Italian autochthonous cultivars reported by Credi et al. (2003) revealed that GFkV was the prevalent virus present in single infections (9.5%), followed by GLRaV-3 (6.3%), GLRaV-1 (2.4%), GVA (2.1%) and GFLV (1.2%). The most common infection with two viruses was a combination of GLRaV-3+GVA (207 vines, 18.6%) and GLRaV-1+GLRaV-3 (61 vines, 5.5%). The most common combinations of three viruses were GLRaV-1+GLRaV-3+GVA (144 vines, 12.9%) and GFkV+GLRaV-3+GVA (66 vines, 5.9%). The prevalent combination of four viruses was GFLV+GLRaV-1+GLRaV-3+GVA (63 vines, 5.6%). Forty-three vines (3.9%) were infected with five viruses with dominant combination GFLV+GFkV+GLRaV-1+GLRaV-3+GVA (19 vines, 1.7%). Three vines were infected with six viruses - two with ArMV+GFLV+GFkV+GLRaV-1+GLRaV-3+GVA and one with GFLV+GFkV+GLRaV-1+GLRaV-3+GVA+GVB. The highest level of mixed infections was determined in the Kaštela region, where some vines showed to be infected with five or even six viruses. This could be due to the limited cultivation area, high populations of insect vectors and/or the usage of infected mother vines for reproduction. Significant number of mixed infections (31%) was also confirmed in Istrian autochthonous cultivars (Poljuha et al., 2010), and a notable proportion of tested vines from the National and Regional collection (Vončina et al., 2011a). A very high mixed infections rate was also found in regions of central and southern Italy (Savino et al., 2001). According to results ratio of vines infected with one or two viruses versus those infected with three to six was 52.4%:47.6%, most similar to results from Calabria region.

The results revealed a highest GLRaV-3 infection incidence in all cultivars, giving it the role of prevalent virus in all surveyed locations with average incidence of 79.6%. Most common was in the Kaštela region where all tested vines of cvs. Babica, Glavinuša, Ljutun, Mladenka, Ninčuša, Dobričić and Vlaška were infected, same as cv. Babić from Primošten region. Results correspond with previously done investigation in Dalmatia (Karoglan Kontić et al., 2009a) and those obtained from National and Regional grapevine collection (Vončina et al., 2011a). In the Istrian peninsula, in two independent surveys, the average GLRaV-3 incidence in autochthonous cultivars was 72.3% (Poljuha et al., 2004) and 61.9% (Poljuha et al., 2010). All mentioned investigations confirm that GLRaV-3 is the most frequently found virus in the Croatian coastal region. High percentage of this virus was also reported in Marocco and Algeria (Digiaro et al., 1999), in many Italian regions, mostly in Calabria and Puglia (Savino et al., 2001), Tunisia (Mahfoudhi et al., 1998), Egipt (Ahmed et al., 2004), in table cultivars from region Vinalopó in Spain (Bertolini et al., 2010). These investigations show that GLRaV-3 is the dominant virus in the Mediterranean region with decreasing frequency northward (Credi and Giunchedi, 1996; Savino et al., 2001; Maixner, 2005).

GVA was the second most widespread virus with average incidence of 61.4%. Since detection was made using monoclonal antibodies probably some strains passed undetected, so actual frequency could be greater than determined. Significant occurrence variation between locations was observed (from 11.1% to 100%) and it

was not found in only 3 locations (two on island Korčula and one in surrounding of Šibenik). On some locations with cv. Plavac mali (data not shown) GVA had higher incidence than GLRaV-3. Up to date the data about GVA in Croatia exist for Istrian peninsula – present in 10.6% (Poljuha et al., 2004), for cultivar Dobričić 18.5% (Vončina et al., 2011b), and in two previously mentioned grapevine collections with incidence of 60% and 32.2%, respectively (Vončina et al., 2011a). The cause of such dispersal may be due to existing planting material, but also presence of insect vectors. In autochthonous cultivars from the Continental region, incidence of GVA is significantly lower – 22.2% (Vončina et al., 2012) with a dispersal effect similar to that of GLRaV-3: decreasing frequency going northward. GVA is relatively widespread in Turkey and Middle Anatolia (Cisgar et al., 2002), on autochthonous cultivar from Greece (Avgelis and Grammatikaki, 2006), in Palestine (Alkowni et al., 1998), Egypt (Ahmed et al., 2004) and Syria (Mslmanieh et al., 2006). Also, it was reported in high frequency from different Italian regions (Savino et al., 2001).

GLRaV-1 was the third most frequently found virus confirmed in 455 vines – 40.8% (Table 1). Compared to previous investigations, GLRaV-1 was found in the Istrian peninsula with an average incidence of 24.3% (Poljuha et al., 2004) and 17.2% (Poljuha et al., 2010). Karoglan Kontić et al. (2009b) reported high infection rates in the autochthonous cv. Žlahtina (60%) typical for Northern Adriatic region and in cv. Kraljevina (38.4%) typical for the Continental region. Relatively high infection rates were also reported from Continental region in cv. Moslavac (32.4%) and in rate of 76% in several cultivars typical for the northern part of Croatia (Vončina et al., 2012). In Slovenia it was found in cv. Refošk with an incidence of 58.6% (Tomažič et al., 2005), while in Italy its occurrence varied from 11.4% in the Ischia region to 63.3% in the Merche region (Savino et al., 2001). From all the mentioned surveys conducted in Croatia and neighboring countries, it is evident that GLRaV-1 infection rate increases from south to north. Also, presence of some insect vector species of GLRaV-1, GLRaV-3 and GVA was reported from Croatia (Maceljski, 1999; Masten Milek, 2007), but their role in spread of viruses under Croatian environmental conditions is still unknown.

The fourth commonly found virus was GFkV (19.9%), prevalent in cvs. Vugava (35.7%), Pošip (33.9%), Babica (33.3%), Mladenka (26.3%) and Babić (23.5%). It was not determined in cvs. Dobričić, Glavinuša and Ninčuša (Table 1). In Croatia GFkV was also reported from Istria in several autochthonous cultivars in rates between 5% and 28.6% (Poljuha et al., 2004), in cv. Plavac mali from collection field in Split (Zdunić et al., 2009) and in the National and Regional collection in 24.2% and 36.8%, respectively (Vončina et al., 2011a). Also, it is one of the most frequent viruses in the Croatian Continental region (Vončina et al., 2012). GFkV frequency is less than reported from central and south Italy (Digiaro et al., 1999), Tunisia (Mahfoudhi et al., 1998), Spain (Fresno et al., 1997) and Serbia (Mandić et al., 2009). It should be pointed out that in this survey monoclonal antibodies were used so possibility that occurrence was underestimated cannot be excluded.

GFLV was most commonly found in cvs. Babica (46.5%) and Plavac mali (31.3%), but not confirmed in cvs. Dobričić, Mladenka and Plavina (Table 1). The average infection rate of 19.6% was higher than determined by Karoglan Kontić et al. (2009a) in autochthonous cultivars included in clonal selection. A higher frequency of this virus was found in 18 autochthonous cultivars in Istria (23.9%), especially in

extensive vineyards (Poljuha et al., 2010) and in cv. Refošk (41.4%) from Slovenia (Tomažič et al., 2005). The presence of GFLV's nematode vector, *Xiphinema index* Thorne, was confirmed in Istrian vineyards (Ivezic et al., 2002) and on islands Vis, Biševo, Korčula and Hvar (Lamberti et al., 1973). Since limited movement of nematodes in soil, infected vines occurred close to each other. This type of occurrence was most evident in one vineyard on the peninsula of Pelješac where symptomatic vines with double nodes and shortened internodes were found next to each other.

The rest of the investigated viruses were found in less than 5% (Table 1). Detailed descriptions, distribution and partial molecular characterization of GLRaV-2 and GVB are previously reported (Vončina et al., 2010; Vončina et al., 2011c).

Conclusions

This investigation confirmed very high virus infection rates in autochthonous grapevine cultivars from Dalmatian region, with GLRaV-3, GVA and GLRaV-1 being the most prevalent viruses. Current sanitary status and viticultural practices (establishing new vineyards by grafting previously planted rootstocks and/or taking buds/scions from the nearest vineyard without appropriate selection) are contributing to the spread of viruses. Also, on market there is an insufficient supply of certified planting material that is available just for few autochthonous cultivars.

According to current "Regulations about market of grapevine planting material" (Official Gazette of the Republic of Croatia, 133/2006), the occurrence of four viruses (ArMV, GFLV, GLRaV-1, GLRaV3) in nurseries must be reduced to a minimum, while in addition rootstocks should also be free from GFkV. Investigation showed high percentage of GVA. Concerning it's proven harmfulness (Goszczynski and Jooste, 2003; Goussard and Bakker, 2006) it would be advisable to include GVA in Croatian certification scheme for grapevine. On the other hand, in the case of very rare and endangered autochthonous cultivars, straightening of certification scheme may further reduce the already limited supply of planting material sources and increase risk of genetic erosion. In this case, a balanced strategy for improving sanitary status must be adopted.

High infection rates of almost all Croatian autochthonous cultivars grown in Dalmatia make their real genetic potential (i.e. berry brix, berry yield, etc.) and breading value unknown and could be the reason why not so amiable to producers. Virus free vines may show better agronomic traits and become of more interest to nurseries and wine/grape producers that will likely have a positive impact on all aspects of Croatian viticultural industry.

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