Sanitary Status of Croatian Native Grapevine Varieties

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Summary

Many grapevine cultivars are grown in Croatia, most of which are considered to be autochthonous. Mass positive selection together with sanitary selection of 15 economically important cultivars was initiated. To this aim, visual observations were made for the presence of virus-induced symptoms. Samples of dormant canes were collected from vines with positive agronomic traits and no visible symptoms of virus infection. The presence of four economically important viruses was evaluated using ELISA (enzyme linked immunosorbent assay): two nepoviruses, *Grapevine fanleaf virus* (GFLV) and *Arabis mosaic virus* (ArMV) and two closteroviruses *Grapevine leafroll-associated virus 1* (GLRaV-1) and *Grapevine leafroll-associated virus 3* (GLRaV-3).

The Survey of virus diseases in commercial vineyards of different vine-growing regions of Croatia disclosed a widespread presence of viruses and a low occurrence of non-infected vines. This is true especially for the coastal region where not a single healthy plant could be found for certain cultivars. Although no visible symptoms were observed, it was not rare to detect multiple infections by two or even three viruses. The most widespread virus in Dalmatia was GFLaV-3 with approximately 80 % of vines infected. In the northern regions almost half of the analysed plants were negative in ELISA and the most represented virus was GLRaV-1. The presence of both nepoviruses is rather low, especially ArMV (only 1 % of vines infected).

Key words

V. vinifera L., native grapevine cultivars, sanitary selection, viruses

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Introduction

So far, a large number of viruses have been detected on grapevine (Martelli and Boudon-Padieu, 2006). Depending on the strain, grapevine cultivar and environmental conditions, virus infections can negatively influence the yield, sugar content and acidity of the must, berry color, resistance to biotic and abiotic stress, length of growing cycle etc. Results from different vine-growing countries show a very high rate of virus-infected vines in some cultivars and production areas (Flak and Gangl, 1994; Kominek and Holleinova, 2003; Lázár, 2003). On virus-infected vines the characteristic symptoms can be observed, although cases exist of latent infections without much symptom expression. Thus much attention has been paid to the sanitary status of plant material. As to varietal improvement, the best results can be achieved if clonal and sanitary selection proceeds together (Goheen, 1989; Maninni, 2000; Mannini, 2003), because virus infection is one of the possible causes of intravarietal morphological variability (Walter and Martelli, 1996; Mannini and Credi, 2000).

Despite the enlarged interest of producers and consumers in Croatian autochthonous varieties, neither systematic clonal selection, nor extensive virus testing of these cultivars, except for some autochthonous cultivars in Istria (Poljuha *et al*., 2004), have ever been done. Thus, to improve the quality and sanitary status of Croatian autochthonous varieties, mass positive selection was initiated and carried out for three years, together with screening for the most important grapevine viruses in all four climatic regions of Croatia. Selection was focused mainly on native cultivars, including the economically important ones and some of the neglected ones, but with high quality potential.

Materials and methods

Twenty one grapevine cultivars growing in Croatia were included in the selection program, identifying stocks with desirable characteristics and without symptoms of virus and virus-like disorders. Virus testing was carried out on 1351 selected vines. Scrapings of phloem tissues, from mature canes collected throughout the dormant season, were homogenized in extraction buffer, pH 8.2 (per 1000 mL deionised water: TRIS 32 g, TRIS-HCl 37.2 g, NaCl 8 g, PVP K25 20 g, PEG-6000 20 g, NaN_3 0.2 g, Tween 20 0.5 mL) in 1/10 ratio. Antiserum kits by BIOREBA (Switzerland) were used for DAS-ELISA identification of *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated virus 1* (GLRaV-1) and *Grapevine leafroll-associated virus 3* (GLRaV-3) (EU directive 2005/43/EC). For all testing, the coating antibodies, samples, controls and conjugate antibodies were incubated for 2.5 h at 37 °C or overnight at 4 °C. Results were read after adding the substrate (*p*-nitrophenyl-phosphate in 10 % diethanolamine, pH 9.8) to the wells. The incubation time for GFLV and GLRaV-3 was 30 to 60 min and for ArMV and GLRaV-1, 60 to 120 min. The presence or the absence of virus was determined by comparing absorbance at 405 nm of the samples with that of the threshold value. Absorbance values greater or lower than the threshold were considered, respectively, as positive or negative results. The threshold was determined as three times the mean absorbance value of the negative control. Positive and negative controls and samples with absorbance values close to the threshold were repeated and interpreted using the data analysis method recommended by BIOREBA.

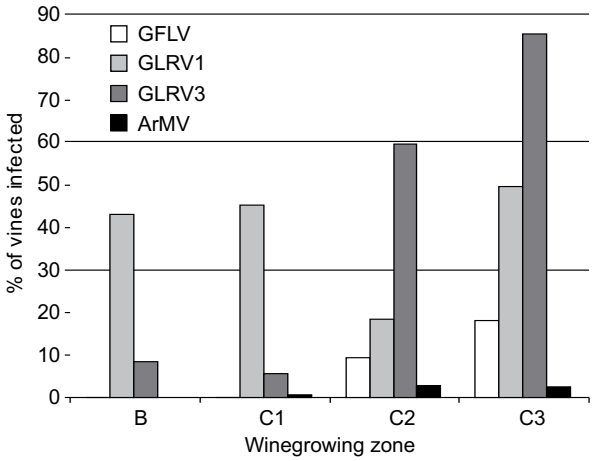
Table 1. List of cultivars, origin of samples and percentage of tested and healthy vines

Cultivar	N° of vines tested	Sub-region/ winegrowing area	Winegrowing zone	Healthy vines/zone
Debit	95	North Dalmatia/ Promina	C3	11 %
Lasina	15			
Plavina	69			
Babica	68	Central and Southern Dalmatia/ Kaštela-Trogir		
Crljenak kaštelanski	16			
Dobričić	3			
Glavinuša	46			
Ljutun	35			
Mladenka	21			
Ninčuša	21			
Vlaška	21			
Malvasija dubrovačka	130	Central and Southern Dalmatia/Konavle		
Plavac mali	122	Central and Southern Dalmatia		
Cipar	11	Croatian Littoral/ Island of Pag	C2	30 %
Gegić	10			
Petovka	11			
Topol	13			
Žlahtina	133			
Graševina	250	Slavonija /Kutjevo	C1	52.04 %
Kraljevina	186	Prigorje-Bilogora	B	50.76 %
Škrlet	75	Moslavina		
Total N° of vines tested	1351			

Results

Results obtained in this research indicate a rather high level of grapevine virus infection in winegrowing regions of Croatia. The problem is especially highlighted in Dalmatia (zone C3), where we have detected only 11% of healthy vines. The situation is slightly better in the northern part of coastal Croatia (zone C2) with about 30% of negative vines, while in the continental winegrowing regions (zone B, zone C) more than 50% of tested vines were negative.

The main grapevine viruses in Croatia are those that cause leafroll disease (GLRaV-1 and GLRaV-3), which generally seem to be the most represented viruses in grapevine (Martelli, 1986; Gugerli, 2003; Credi and Giunchedi, 1996; Maixner, 2005). GLRaV-1 prevailed in the continental regions, while 86 % of tested Dalmatian vines were positive for GLRaV-3. These data tally with the notion that GLRaV-3 is more common in the Mediterranean (Cabaleiro and Segura, 2006; Ahmed *et al*., 2004) and GLRaV-1 in the northern viticultural regions



Picture 1.
Geographic distribution of grapevine viruses in Croatia

of the world (Credi and Giunchedi, 1996; Savino *et al*, 2002; Maixner, 2005). Although spread of infected plant material is assumed to be the principal means of dissemination, in some Mediterranean and overseas countries, the spreading at a site of GLRV-3 associated with insect vectors (*Pseudococcidae* and *Coccidae*) has been reported (Cabaleiro and Segura, 1997; Sforza *et al*., 2003). The incidence of nepoviruses (GFLV and ArMV) in Croatia is significantly lower. In continental vine-growing areas infection with GFLV is very rare since out of 511 tested vines only two were positive. In zone C2 and C3 (Mediterranean region) GFLV was found in about 16 % of the vines. The least represented virus (out of the four tested) was ArMV, detected only in 23 cases, among which only two were sampled in continental vineyards.

The multiple infections with two or more viruses are quite common. The most common combination is infection with LR1 GLRaV-1 and GLRaV-3, which is the case in 25 % of all vines tested, but in more than 50 % of Dalmatian vines. In this region (zone C3), a further 10 % of tested vines are infected with GFLV besides the GLRaV-1 and GLRaV-3 viruses. Multiple infections other than the LR1 and LR3 combination is very rare in the continental part of Croatia and it has been observed in only two out of the 511 vines tested.

In this survey cultivars with different population size and economical importance have been investigated. This is why the numbers of tested vines vary greatly. 'Graševina', 'Kraljevina', 'Plavac mali' and 'Žlahtina' have a rather large population and are economically significant cultivars in their winegrowing regions. The results show that enough healthy vines can be found (more than half of all tested) of 'Graševina' and 'Kraljevina', as a basis for the further clonal selection procedure, without concern for intravarietal variability loss. The situation is also favourable for 'Žlahtina' where nearly 40 % of vines were negative in ELISA. According to our findings the situation is very serious in the population of 'Plavac mali', where only seven out of 122 tested vines were negative in ELISA. In this population all four viruses can be found. More than 50 vines were infected with two viruses (the most common combination is GLRaV-1 and GLRaV-3), 26 vines were infected with three viruses (usually GFLV, GLRaV-1 and GLRaV-3), and one with all four viruses. 'Plavac mali' is the most important red wine variety in Croatia, having a

Table 2. Incidence of cultivars infection with different viruses and virus combination

Cultivar	% of vines infected with									Winegrowing zone
	LR1	LR3	GFLV	ArMV	LR1 + LR3	GFLV + LR1 + LR3	GFLV + LR3	GFLV + ArMV + LR1 + LR3	ArMV + LR1 + LR3	
Crljenak kaštelanski					50	43.7		6.3		C3
Babica		1.5			84	7.4			5.9	
Debit	5.3	56.8			18.9					
Dobričić		66.6			33.4					
Glavinuša					52.2	39.1		4.3	4.3	
Mladenka		28.6				19	47.6			
Lasina	13.3	33.3			46.7					
Ljutun					88.6	11.4				
Malvasija dubrovačka		3.8			93.8	0.8				
Ninčuša		57.1			14.3	19	9.5			
Plavac mali	1.6	27.9	1.6		19.7	18	21.3	0.8	0.8	
Plavina	1	21.7			1.5					
Vlaška		66.7			9.5		23.8			
Žlahtina	60	1.3			5.3		9			C2
Cipar	26.7	2.2			90.9					
Gegić	7.5	31.6	3	2.3						
Petovka					36.4					
Topol		100			7.7		7.7			
Graševina		63.6			2.4					C1
Škrlet		84.6			16	1.3				B
Kraljevina	38.4	3.2		0.8	2.2					
Total	16.1	17.1	0.4	0.37	25.3	4.9	4.2	0.3	0.5	

phenotypically heterogeneous population widespread in the entire coastal region, so that the risk of loosing intravarietal variability after completing clonal selection is reasonable.

The obtained results show that cultivars with very low population, grown in one narrow viticultural area are usually almost completely virus infected ('Mladenka', 'Vlaška', 'Ljutun', 'Ninčuša', 'Glavinuša', 'Cipar', 'Gegić', 'Topol' etc.). The level of virus infection varies also between winegrowing areas. For example in the Kaštela -Trogir winegrowing area we could not find a single healthy vine or meter of cultivar. Every vine of 'Ljutun', 'Glavinuša', 'Ninčuša', 'Vlaška' and 'Crljenak kaštelanski' was infected with GLRaV-3 virus. Beside the GLRaV-3, vines are often infected with other viruses (usually GLRaV-1), but in this region a rather high infection was observed with GFLV, too (39 % of 'Glavinuša' vines, 19 % of 'Ninčuša' vines and 11.4 % of 'Ljutun' vines were infected with all three viruses). Grenan *et al.* (2000) reported a similar situation with some minor cultivars at the island of Corsica in France. Despite the high quality potential of these cultivars the sanitary status of their population is serious and threatens their complete loss. In order to preserve them and return them to production it is necessary to create the preconditions for certified "virus-free" plant material production. As long as the chance of finding healty vines in their population is negligible it will be necessary to undertake their sanitation through tissue culture and heat therapy.

In contrast to Kaštela, in the Promina winegrowing region we detected a much lower level of virus infection in general, and especially with cv. Plavina (67 % of negative vines). On most of the infected vines the GLRaV-3 virus was detected and in some cases vines were infected with GLRaV-1 virus, while no nepoviruses were detected in this area. For these cultivars the proportion of ELISA negative vines in the population would probably be high enough for subsequently proceeding with selection and preservation of intravarietal variability.

Conclusions

1. Among Croatian grapevine varieties a high level of vines infected with the four most economically important viruses has been detected.
2. In Dalmatia (zone C3) only 11 % of ELISA negative vines were found, in the northern part of the coastal region (zone C2) 30 %, while in continental Croatia (zone C1 and B) more than 50 % of ELISA negative vines were found.
3. The Most common viruses are GLRaV-3 (Zone C_2 and C_3) and GLRaV-1 (Zone C1 and B). In Dalmatia almost all infected vines are positive on GLRaV-3 and 45 % of them on GLRaV-1, too.
4. The incidence of nepovrius (GFLV and ArMV) is significantly lower, especially in continental Croatia, while in the coastal region 16 % of vines were infected (mostly with GFLV).
5. In some narrow winegrowing areas (Kaštela-Trogir) with local, small population varieties no ELISA negative vines were found and for further propagation it will be necessary to undertake their sanitation through tissue culture and heat therapy.
6. Among economically important cultivars, with big populations, like Graševina, Kraljevina and Žlahtina the proportion of ELISA negative vines ensures a good base for individual clonal selection, while for Plavac mali a very small number of non infected vines detected is a serious risk for loosing intravarietal variability after completing selection. Therefore it will probably be necessary to undertake sanitation of this cultivar.

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