Ampelographic Description and Sanitary Analysis of Four Istrian Grapevine Varieties (*Vitis vinifera* L.)

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Summary

Istrian Peninsula, one of the five districts within viticultural region of Coastal Croatia, provides great geological-reliefal and climatic diversity and various production conditions. This research studied the autochthonous varieties and their sanitary status in old vineyards. Considering the age of vineyard, ten locations were chosen where four autochthonous varieties 'Malvasia istarska', 'Teran', 'Borgonja', and 'Pergola velika' were identified using ampelographic description according to OIV descriptors. Morphological characteristics of chosen varieties were described using OIV parameters and must was chemically analysed (pH value, sugar content, titratable acidity). High intra cultivar variability was found for weight of a single bunch especially for 'Teran'. There were also differences in sugar content of must particularly for 'Pergola velika'. Must pH was low for all varieties with predominantly low acidity value. Sanitary status of vines was determined by testing the plant samples for the presence of three grapevine viruses (GLRaV-1, GLRaV-3 and GFLV) using DAS-ELISA. The percentage of infection for GFLV was 55.6% while for GLRaV-1 and GLRaV-3 it was 61.1%. Results showed that some morphological characteristics differ from characteristics described in literature. With purpose of preserving the biodiversity of autochthonous varieties and for future researches, healthy propagation material will be collected and planted in collection field of autochthonous varieties at the Institute of Agriculture and Tourism, Poreč.

Key words

biodiversity, autochthonous varieties, sanitary status, morphological characteristics

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Introduction

Croatia is, for its geographic and climatic position one of the most biodiverse countries in Europe. Istrian Peninsula, placed in the western part of Croatia and within 2820 km^2, provides great geological-reliefal and climatic diversity. On relatively small territory of Istria, very different, even opposite natural-production conditions are provided (Vitolović, 1960). In viticultural sense, Istria, as one of the five districts within viticultural region of Coastal Croatia, has major part in Croatian viticulture (NN 159/2004). Grapevine (*Vitis vinifera* L.) is species known for its great cultivar and intercultivar diversity. There are a few papers involved with vine assortment in Istria: complete (Vitolović, 1960) and autochthonous (Staver and Peršurić, 2002). 'Malvazija istarska' is the most important white cultivar in Istria. Other important white cultivars are 'Chardonnay', 'Pinot blanc', 'Pinot gris', 'Sauvignon blanc' and 'White Muscat'. Most common red varieties are 'Teran', 'Borgonja', 'Merlot', 'Hrvatica', 'Cabernet Sauvignon', 'Cabernet franc' and 'Pinot noir' (Maletić et al., 2008). Some of those cultivars have been researched by scientists from Institute of Agriculture and Tourism within scientific projects "Genetic and economic resources *Vitis* sp." (2001-2006), "Valorization of grapevine (*Vitis* sp.) resources and gene bank" (2006-2011) and long-term studies of autochthonous Istrian cultivar biodiversity, following the global trend in viticulture preserving, based on autochthonous and typical cultivars in particular vinegrowing regions (Peršurić et al., 2004; Sladonja et al., 2004; Sladonja et al., 2005; Ilak Peršurić et al., 2006; Pribetić et al., 2006). Besides the most common varieties in Istria, a rare and almost extinct variety Jarbola was also investigated within those studies. Ampelographic and molecular characterization proved Jarbola most likely to be autochthonous cultivar (Sladonja et al., 2007).

Former researches have determined that GLRaV-3, GLRaV-1 and GFLV are the most spread viruses among autochthonous Istrian cultivars with frequencies of infection of 72.3% for GLRaV-3, 23.9% for GFLV and 24.3% for GLRaV-1 (Poljuha et al., 2004; Poljuha et al., 2010). That is the reason why this research is focused just on these viruses. Basic symptoms of *Grapevine leafroll-associated virus* (GLRaV) are change of fruit colour (red cultivars), fruit ripening delay and yield decrease (Martelli, 1993). *Grapevine fanleaf virus* (GFLV) can cause stunted growth, poor fruit quality, yield loss and plant lodging (Martelli and Savino, 1990).

The aim of this research was to prevent the loss of old autochthonous Istrian varieties by planting an Institute collection plantation. Also, to point out the value of production characteristics of autochthonous varieties that have been lost during modernization and commercialization of Istrian viticulture by determine the range of morphological, sanitary and ampelometric diversity of studied varieties (Zdunić et al., 2007). Determination of vine sanitary status (ELISA-test) helps in reduction of grapevine infection with viruses, so quality plant material for future propagation can be produced (Ministry of Agriculture, Fishery and Rural Developement, 2006).

Materials and methods

Ampelographic analysis

Plant material for ampelographic description and chemical analysis was collected in September 2009 at usual harvest time in 10 vineyards older than 50 years: Badnjevari, Benazići, Trkusi, Tončinići, Perkovići, Paljuhi, Sovinjak, Sovinjak-S. 1, Sovinjak-S. 2 and Peršurići. Selection criteria were the age of vineyard and the assortment. With the age of vineyard as a limiting factor, only 10 vineyards were found for this study. Vines were chosen by visual detection and only visually healthy vines were taken. There have been selected 46 vines of 'Teran', 39 vines of 'Malvazija istarska', 12 vines of 'Borgonja' and 12 of 'Pergola velika' (Table 1).

Ampelographic analyses were done using OIV descriptors, modified by the European Union Project GENRES 081 (2001). Description of the different parts of the varieties followed the methodologies reported in each OIV descriptor (OIV descriptors, 2001). The following descriptors for bunch at harvest ampelographic description were used: OIV 202 bunch length; OIV 204 bunch density; OIV 206 length of peduncle; OIV 208 bunch shape; OIV 209 number of wings; OIV 220 berry length; OIV 221 berry width; OIV 223 berry shape; OIV 225 colour of berry skin; OIV 230 color of berry flesh; OIV 235 degree of firmness of berry flesh; OIV 236 berry particular flavour; OIV 241 presence of seeds in berry; OIV 502 weight of single bunch and OIV 503 single berry weight. The results of must chemical analysis were determined by three OIV descriptors: 505 sugar content of must; 506 total acids content of must and 508 pH of must.

Total must sugar content was measured by digital refractometer (Artisan TM HR200, Canada), total must acids content was measured by titration with 0.1 N NaOH and must pH with digital pH meter (Mettler Toledo MP220, Germany).

Sanitary status

Plant tissue samples from 36 vines were analysed for three economically most important viruses: *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated virus 1* (GLRaV-1) and *Grapevine leafroll-associated virus 3* (GLRaV-3) (Poljuha et al., 2004; Poljuha et al., 2010; NN 133/2006). Commercial kits for DAS-ELISA (Neogen Europe Ltd, Scotland) were used according to the manufacturer's instructions.

Results and discussion

Ampelographic analysis

Obtained results using OIV descriptors for each variety are shown in Table 1. Ampelographic analysis of 46 individual vines of 'Teran' showed some differences from the literature: for OIV code 206 the most frequent petiole length was short, while in literature petiole length is described as medium (Mirošević and Turković, 2003); for OIV code 220, the most common berry length was medium; for OIV code 221, the most common berry width was medium, literature describes berry as medium or large (Mirošević and Turković, 2003); for OIV code 223, the most frequent berry shape was roundish while literature describes berry as ovate (Mirošević and Turković, 2003); for OIV code 230, the

Table 1. Ampelographic characterization of four investigated grapevine varieties

OIV Code	Characteristic	Mean value with standard deviation (for quantitative variables); Most frequent notation (description)			
		Malvazija istarska n=39	Teran n=46	Borgonja n=12	Pergola velika n=12
			Bunch		
202	Length (cm)	20.1±1.9 7 (long)	18.6±3.06 5 (medium)	5 (medium)	7 (long)
204	Density	5 (medium)	5 (medium)	3 (loose)	7 (dense)
206	Length of peduncle (cm)	4.9±0.9 3 (short)	3 (short)	1 (very short)	3 (short)
208	Shape	1 (cylindrical)	2 (conical)	2 (conical)	2 (conical)
209	Number of wings	2 (1-3 wings)	2 (1-3 wings)	2 (1-3 wings)	2 (1-3 wings)
502	Weight of a single bunch (g)	218.7±70.2 3 (low)	290±116.2 3 (low)	141.67±51.5 1 (very low)	391.67±90.0 5 (medium)
			Berry		
220	Length (mm)	16.4±2.0 5 (medium)	5 (medium)	3 (short)	5 (medium)
221	Width (mm)	16.4±2.0 5 (medium)	3 (small)	3 (small)	5 (medium)
223	Shape	2 (roundish)	2 (roundish)	2 (roundish)	3 (elliptic)
225	Color of skin	1 (green-yellow)	6 (blue-black)	6 (blue-black)	1 (green-yellow)
230	Color of flesh	1 (not colored)	1 (not colored)	1 (not colored)	1 (not colored)
235	Degree of firmness of flesh	1 (very soft)	1 (very soft)	1 (very soft)	5 (medium)
236	Particular flavour	1 (none)	1 (none)	4 (herbaceous)	1 (none)
241	Presence of seeds	3 (present)	3 (present)	3 (present)	3 (present)
503	Single berry weight (g)	2.57±0.79 3 (low)	3.53±0.78 5 (medium)	1.58±0.90 1 (very low)	3.92±0.29 5 (medium)

most common color of berry flesh was not coloured, in literature, colour of berry flesh is described as reddish (Mirošević and Turković, 2003); for OIV code 235, the most frequent degree of firmness of berry flesh was very soft, while literature describes 'Teran' flesh as crispy (Mirošević and Turković, 2003).

Ampelographic analysis of 39 vines of 'Malvazija istarska' showed the following differences from literature: for OIV code 202, the most common bunch length was long (20.1 cm), literature describes medium bunch length (Vitolović, 1960); for OIV code 206 the most frequent petiole length was short (4.9 cm), in literature petiole length is described as long and thick (Vitolović, 1960); for OIV code 235, the most frequent degree of firmness of berry flesh was very soft, literature describes 'Malvazija istarska' flesh as very firm (Vitolović, 1960).

The results of the present study were also compared to European *Vitis* Database (http://www.eu-vitis.de) where different data for the following characteristics were found: length of peduncle (long, ~9 cm), bunch shape (conical) and total acidity of must (medium, 8.25 g/L). For the weight of a single bunch (OIV 502), there was no data found.

Ampelographic analysis of 12 vines of 'Borgonja' also showed some differences from the literature: for OIV 204, the most common bunch density was loose. Ampelographic atlas describes 'Borgonja' bunch as very dense (Mirošević and Turković, 2003); for OIV code 208, the most frequent bunch shape was conical, literature describes 'Borgonja' bunch as cylindrical-conical (Mirošević and Turković, 2003); for OIV code 220, the most common berry length was short; for OIV code 221, the most common berry width was small, literature describes berry as medium (Mirošević and Turković, 2003); for OIV code 236, the most common berry particular flavour was herbaceous, literature says that berry has no particular flavour (Mirošević and Turković, 2003).

In literature, there was no data found for 'Pergola velika'. Our results for all OIV codes measured were as follows: for OIV code 202, the most common bunch length was long; for OIV code 204, the most common bunch density was dense; for OIV code 206 the most frequent petiole length was short; for OIV code 208, the most frequent bunch shape was conical; for OIV code 209, the most represented number of wings was 1-3; for OIV code 220, the most common berry length was medium; for OIV code 221, the most common berry width was medium; for OIV code 223, the most frequent berry shape was elliptic; for OIV 225, the most common color of berry skin was green-yellow; for OIV code 230, the most common color of berry flesh was not coloured; for OIV code 235, the most frequent degree of firmness of berry flesh was medium; for OIV code 236, the most common berry particular flavour was none; for OIV code 241, the most frequent presence of seeds in berry was present; for OIV 502, the most common weight of single bunch was medium (391.67 g); for OIV 503, the most common single berry weight was medium (3.92 g).

Table 2. Chemical characteristics of must

OIV Code	Characteristic	Mean value with standard deviation; Most frequent notation (description)			
		Malvazija istarska n=39	Teran n=46	Borgonja n=12	Pergola velika n=12
505	Sugar content of must (°Brix)	19.53±2.37 7 (high)	16.80±1.63 5 (medium)	20.42±1.43 7 (high)	14.66±2.94 1 (very low)
506	Total acids content of must (g/l TA)	5.66±1.19 3 (low)	11.97±1.86 7 (high)	6.58±1.65 3 (low) and 5 (medium)	6.98±1.14 3 (low)
508	pH of must	3.36±0.11 3 (low)	2.98±0.13 3 (low)	3.22±0.25 3 (low)	3.17±0.17 3 (low)

Chemical analysis of must

Results obtained using OIV parameters for must chemical analysis are shown in Table 2.

'Terans' total sugar content (°Brix) varied from 13.30 to 20.30 with mean value 16.80; total acids content (g/l) varied between 7.70 and 15.50 with mean value 11.97; pH value varied between 2.74 and 3.33 with mean value 2.98.

'Malvazija istarska' had total sugar content (°Brix) from 15.30 to 23.90 with mean value 19.53; total acids content (g/l) varied between 4.20 and 8.50 with mean value 5.66; pH value varied between 3.10 and 3.56 with mean value 3.36.

Total sugar content (°Brix) for 'Pergola velika' varied from 10.80 to 19.60 with mean value 14.66; total acids content (g/l) varied between 5.60 and 9.20 with mean value 6.98; pH value varied between 2.98 and 3.40 with mean value 3.17.

'Borgonja' had total sugar content (°Brix) from 17.30 to 22.90 with mean value 20.42; total acids content (g/l) varied between 3.50 and 8.40 with mean value 6.58; pH value varied between 3.00 and 3.76 with mean value 3.22.

Sanitary status

The distribution of virus infection among autochthonous grape varieties in Istria is presented in Table 3. According to our research the most widespread viruses were GLRaV-1 and GLRaV-3 (61%), while the distribution of GFLV was slightly lower (56%). Prevoius surveys on Istrian varieties found out incidence of 69.1-72.3% for GLRaV-3; 17.2-24.3% for GLRaV-1 and 14-23.9% for GFLV (Poljuha et al., 2004; Poljuha et al., 2010). 'Borgonja' showed very variable infection for three viruses (from 17% for GFLV to extremely high 100% for GLRaV-1 and GLRaV-3). Like this, GLRaV-3 was the most prevalent virus and its incidence for 'Borgonja' was also 100% (Poljuha et al., 2004). Virus incidence in our survey for 'Teran' was 91% compared to previous survey 66.1%, for Borgonja the percentage of infection was 100% compared to previous 95.8% (Poljuha et al., 2010). No variety was free from investigated viruses and multiple infections were found in all four investigated cultivars.

Table 3. The distribution of viruses among autochthonous grape varieties in Istria

Variety	No. of samples tested	Percentage of samples infected (%)	No. of infected vines and percentage of infection (in parenthesis)	
			GFLV	GLRaV-1, 3
Teran	11	91	7 (64)	5 (45)
Malvazija istarska	11	73	7 (64)	5 (45)
Pergola velika	8	88	5 (63)	6 (75)
Borgonja	6	100	1 (17)	6 (100)
Total	36	-	20 (55.6)	22 (61.1)

Conclusion

Analysis of given data showed intercultivar diversity. On the other hand, some of the codes showed complete uniformity for every cultivar: OIV code 230 (colour of berry flesh), OIV code 241 (presence of berry seeds) and OIV code 508 (pH of must). For other OIV indicators, higher or smaller diversity was determined.

Vine sanitary status analysis showed high infection level of old autochthonous grape varieties in Istria with three economically most important viruses: GFLV, GLRaV-1 and GLRaV-3. That indicates the necessity of better forthcoming vine sanitary selection in old vineyards.

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