AMPELOGRAPHIC AND OENOLOGICAL CHARACTERIZATION OF THE ANCIENT GRAPEVINE CV. DOBRIČIĆ GROWN IN THE COASTAL REGION OF CROATIA

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Abstract

Aims : A four-year study (2007 through 2010) was carried out to determine agronomic, biological, and oenological characteristics of Dobričić grapevines so as to evaluate this cultivar and protect it from extinction.

Methods and results : 38 characteristics from the OIV descriptor list were used to describe young shoot, mature leaf, cluster, and berry morphology of Dobričić grapevines. The dimensions of an average leaf were constructed from measured leaf variables. A typical Dobričić genotype at nine microsatellite loci is presented. ELISA analysis revealed high virus incidence and all samples tested were GLRaV-3 positive. The profile of different classes of polyphenols in Dobričić wine was determined using HPLC and spectrophotometry. The concentrations of polyphenols in Dobričić wine, especially anthocyanins, hydroxycinnamic acids and stilbenes, were high compared to major red wines reported in the literature. Descriptive sensory analysis was carried out to determine the aroma attributes describing a Dobričić monovarietal wine.

Conclusion : Knowledge of the biodiversity of the grapevine cultivars of Croatia is still scant. This study provides for the first time ampelographic and oenological information on Dobričić grapevines and demonstrates its high quality potential.

Significance and impact of the study : The results of the ampelographic characterization would be helpful in the identification and selection of Dobričić for cultivation in certain vine-growing areas. The outstanding quality of Dobričić makes it very promising for wine blending (colour improvement) and plant breeding purposes.

Key words : ampelography, Dobričić, biodiversity, agronomic trait, polyphenolic profile

Résumé

Objectifs : Une étude sur quatre ans a été menée (de 2007 à 2010) sur des vignes de Dobričić pour en déterminer les caractéristiques agronomiques, biologiques et œnologiques, afin d'évaluer ce cultivar et de le protéger de l'extinction.

Méthodes et résultats : 38 critères de la liste de descripteurs de l'OIV ont été utilisés pour décrire la jeune pousse, la feuille mature, la grappe et la morphologie des baies du cultivar Dobričić. Des mesures effectuées sur un échantillon de feuilles ont permis de définir les dimensions d'une feuille moyenne. En utilisant neuf loci de microsatellites, un génotype standard pour Dobričić est présenté. Des analyses ELISA ont révélé l'incidence élevée de virus et tous les échantillons testés étaient positifs pour GLRaV-3. Le profil de différentes classes de polyphénols dans le vin issu de Dobričić a été déterminé par HPLC et analyse spectrophotométrique. Il a été trouvé que la concentration de polyphénols dans le vin, en particulier en anthocyanes, en acides hydroxycinnamiques et en stilbènes, était élevée en comparaison de la plupart des vins rouges décrits dans la littérature. L'analyse sensorielle descriptive a été effectuée afin de déterminer les attributs de l'arôme qui caractérisent le vin monovariétal Dobričić.

Conclusion : La connaissance de la biodiversité de cultivars de vigne en Croatie est encore insuffisante. La présente étude fournit pour la première fois des informations ampélographiques et œnologiques sur le cépage Dobričić, et démontre son haut potentiel pour des vins de qualité.

Signification et impact de l'étude : Les résultats de la caractérisation ampélographique pourraient être utiles pour identifier et sélectionner Dobričić afin de le cultiver dans certaines régions viticoles. Les paramètres de qualité exceptionnelle de Dobričić constituent des indices très prometteurs vis-à-vis de perspectives d'assemblages, mais aussi de croisements.

Mots clés : ampélographie, Dobričić, biodiversité, critères agronomiques, profil polyphénolique

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INTRODUCTION

Dobričić is an old native Croatian grapevine cultivar (*Vitis vinifera* L.). It is grown in the coastal region and islands of Dalmatia in southern Croatia. Currently, it is not widely grown and the highest number of Dobričić old vine representatives can be found on Šolta Island. Dobričić is cited in literature dating from the early 19th century as being grown in the Dalmatian region (RITTER VON HEINTL, 1821). At the time of the *phylloxera* crisis in Europe, wines produced from Dobričić were highly esteemed and exported to France because of its intense red colour and high extract content (BULIĆ, 1949). However, to date there is no ampelographic description of Dobričić and literature data regarding its agronomic and oenological characteristics is scant. According to the grape growers from Šolta Island, Dobričić is a low-yielding cultivar, but it shows more resistance to diseases than other Croatian cultivars. Currently, there are no Dobričić wines available on the market.

Old native cultivars are very valuable sources of genetic variability and can be very important for the production of high-quality wines. To characterize the agronomic and biological value of cultivars, it is necessary to analyze various factors. As emphasis has been placed on the evaluation of wine quality, micro-scale vinification has been integrated into many grapevine selection programs.

Besides conventional ampelography methods, one of the most useful tools to characterize the value of grapevine cultivars is a large group of polyphenolic compounds. Of special interest for the quality of red wine are flavonoids: anthocyanins, flavonols, flavan-3-ols and proanthocyanidins (condensed tannins), which normally make up 80 to 90 % of the polyphenolic content of red wine (MAZZA, 1995; KALLITHRAKA *et al.,* 2006; MARKIS *et al.,* 2006), while non-flavonoids make up only a smaller part of it, including phenolic acids and their derivatives, phenolic alcohols and stilbenes.

Recently, Croatia has launched a project to identify and evaluate native grape cultivars. After several years of *in situ* evaluation, many cultivars were propagated in a grape germplasm repository to describe their properties and conserve genetic diversity. Some of the minor grapes from the Dalmatian region, like Dobričić, are in danger of extinction.

The aim of this study was to assess the ancient grapevine cultivar Dobričić (*Vitis vinifera* L.) and to provide ampelographic, agronomic and oenological descriptions of this cultivar to prevent its extinction.

MATERIALS AND METHODS

1. Plant material

The study was carried out from 2007 to 2010 on *Vitis vinifera* L. cv. Dobričić vines grafted onto *Rupestris du Lot* rootstock and grown in a 60-year-old vineyard on Šolta Island (Central Dalmatia, Croatia) (Fig. 1). Row and vine spacing was 1.2 × 1.0 m. Vines were trained to a *gobelet* system and pruned to 8 to 10 two-node spurs per vine. The vineyard was managed according to the standard viticultural practice for the region. No irrigation was applied during the four-year evaluation period. The soil in the vineyard was a red Mediterranean soil known as *terra rossa*. Central Dalmatia is considered to have a Mediterranean climate.

2. Ampelographic characterization

Observations were made four times per season, at the phenological stages of shooting, flowering, veraison and ripening. The observed descriptors correspond to the OIV descriptor list modified by the European Union Genres 081 project (2001). A total of 38 descriptors were included, including young shoot, mature leaf, inflorescence, and cluster and berry morphology.

Between fruit set and veraison, a representative sample of 10 fully grown leaves was taken from the middle third of a shoot. Leaves were photographed with a digital camera (Olympus, model SP-500UZ), and the Superampelo computer program (SOLDAVINI *et al.* 2009) was used to measure the leaf variables which were used to construct the shape and size of an average Dobričić leaf.

3. Microsatellite analysis

The microsatellite analysis was performed as a complementary method to standard ampelographic



Figure 1 - Appearance of Dobričić grape cluster and leaf. Photo taken in 2008 at a commercial vineyard on Šolta Island.

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evaluation to confirm that investigated vines were true-to-type. Young leaves were collected in the spring from shoot tips of individual vines, freeze dried, and DNA was extracted using CTAB solution as described (DOYLE and DOYLE, 1987). Nine polymorphic microsatellite loci were analyzed : VVMD5, VVMD7 (BOWERS *et al.,* 1996), VVMD25, VVMD27, VVMD28, VVMD32 (BOWERS *et al.,* 1999), VVS2 (THOMAS and SCOTT, 1993), VrZAG62, and VrZAG79 (SEFC *et al.,* 1999). These nine markers were used by the GrapeGene06 project (http://www1.montpellier.inra. fr/grapegen06/accueil. php) as a standard international set for grapevine characterization and are used in the European *Vitis* Database (EVD, http://www.eu-vitis.de).

DNA was PCR-amplified in a 15-µL reaction mixture containing 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2 mM MgCl2 (all included in 10 µL 109 PCR buffer), 10 pmol of each primer, 200 lM of each dNTP, 2 U Taq polymerase (Perkin Elmer Biosystems, California, USA), and 50 ng template DNA. Four primer pairs were used in each reaction to allow multiplexing. The PCR conditions were as follows : 1 cycle of 94ºC for 5 min ; 30 cycles of 94ºC for 30 sec, 55ºC for 30 sec, and 72ºC for 40 sec ; and then a final elongation cycle of 72ºC for 7 min. Amplified products were resolved using capillary electrophoresis on a 3130xl Genetic Analyzer with the Data Collection software, version 1.2 (PE/Applied Biosystems). The data were further analyzed using Genescan Version 3.1 and Genotyper Version 2.5. Gained values were compared to a genotype for Dobričić in the Grape DNA Reference SSR database maintained by the European *Vitis* Database.

4. Agronomic characteristics

Grapes from 10 individual vines were harvested at technological maturity, which was fixed according to the established vintage schedule in the area. All vines were harvested on the same date, although harvest dates differed each year : 13 September 2007, 18 September 2008, 15 September 2009 and 24 September 2010. Clusters per vine were counted and the yield of each of 10 individual vines was measured. Ten clusters were randomly collected and cluster weight (g), length (cm) and width (cm) were measured. Number of berries was counted for each representative cluster and berry weight was determined.

5. Grape maturity parameters

100-berry samples of each vine were manually crushed and basic must composition was measured. Sugar content (ºBrix) was measured using a temperature-compensating refractometer (Atago, model WM-7). Total titratable acidity (g/L) was measured by titrating with 0.1 M NaOH and bromothymol blue as indicator. Juice pH was measured with a pH meter (Methrom, model 719 S).

6. Polyphenolic content from grape seed and skin extract

Three representative samples (9 kg) were selected at ripening during the 2007 and 2008 harvests. From the clusters, 200 g berries from each sample were randomly selected. Grape extracts were prepared according to MATTIVI *et al.* (2002). The seeds and skins were manually separated from the berries and put into an ethanol/water solution (12 : 88 v/v) with 50 mg/L SO_2 and 5 mg/L tartaric acid, neutralized with 1/3 M NaOH to pH=3.2. Extraction lasted for five days at 30 °C. After extraction, the solution was separated from the solids and then used for the analysis of total phenols, total anthocyanins, vanillin index and proanthocyanidins.

Total phenolic content (TPC) was measured by the Folin-Ciocalteu method (SINGLETON and ROSSI, 1965 ; RIGO *et al.,* 2000). Total anthocyanins (TA) were determined according to the method of RIGO *et al.* (2000). Concentrations of compounds defined by vanillin index (catechins and low-molecular mass proanthocyanidins) (VAN) were obtained by optimizing the conditions as described by RIGO *et al.* (2000). Proanthocyani-dins (high-molecular mass molecules, PA) were determined through transformation into cyanidin (RIGO *et al.* 2000). Analysis was done by Varian Cary 50 spectrophotometer (double-beam).

7. Sanitary analyses

The cuttings of 10 individual vines collected from the investigated vineyard during dormancy were analyzed by several enzyme-linked immunosorbent assay (ELISA) protocols for virus detection : double antibody sandwich ELISA (DAS-ELISA) for *Grapevine fanleaf virus* (GFLV), *Arabis mosaic virus* (ArMV), *Grapevine leafroll-associated virus types 1* and *3* (GLRaV-1 and GLRaV-3), double antibody sandwich indirect ELISA (DASI-ELISA) for *Grapevine fleck virus* (GFkV) and protein A double antibody sandwich ELISA (protein A-DAS-ELISA) for detection of *Grapevine virus A* (GVA). All reagents were provided by Agritest (Valenzano-Bari, Italy) and used according to the manufacturer's instructions.

8. Experimental winemaking

All grapes from the vineyard on Šolta Island (~ 60 kg) were retained for winemaking in 2008 to produce varietal wine. Because of the relatively small amount of grapes, fermentation was performed without replicates. Grapes were destemmed, crushed, sulfited to 25 mg/L free sulfur dioxide (SO_2) and stored in a 70-liter stainless steel vat. Micro-scaled fermentation was carried out using the commercial strain EC1118 (*Saccharomyces bayanus*, Lallemand Inc, Canada). The cap was punched down

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twice a day until it remained submerged during a six-day maceration. The pomace was pressed and fermentation was completed in stainless steel at 20 °C. At the completion of fermentation, the wine was racked and sulfited to 50 mg/L (SO_2). The second racking was done in April and the wine was finally bottled after correction of free SO_2 to 20-30 mg/L. Malolactic fermentation was not performed. The wine was analyzed for classical parameters in duplicate following the standard methods recommended by the O.I.V. (2000), two months after bottling.

9. Polyphenolic content in wine

Data about polyphenols in Dobričić wine are not available yet. To get a preliminary estimate of the types and concentrations of polyphenols in wine produced from this cultivar, analysis of different classes of polyphenols

Table 1 - OIV characters for the description of young shoot, mature leaf, cluster and berry in Dobričić.

OIV Code	Characteristic	Level of expression
OIV-001	Young shoot: shape of the tip	7 (opened)
OIV-003	Young shoot: intensity of anthocyanin coloration on prostrate hairs of the tip	1 (absent or very weak)
OIV-004	Young shoot: density of prostrate hairs of the tip	3 (sparse)
OIV-007	Shoot: colour of dorsal side of internodes	7 (green)
OIV-008	Shoot: colour of ventral side of internodes	7 (green)
OIV-015-1	Shoot: distribution of the anthocyanin coloration on the bud scales	1 (absent)
OIV-015-2	Shoot: intensity of anthocyanin coloration on the bud scales	1 (absent or very weak)
OIV-016	Tendrils: distribution on the shoot	1 (discontinuous)
OIV-051	Young leaf: colour of the upper side (leaf 4)	4 (copper - reddish)
OIV-053	Young leaf: density of prostrate hairs between veins at the lower side of leaf	3 (weak)
OIV-067	Mature leaf: shape of blade	3 (pentagonal)
OIV-068	Mature leaf: number of lobes	3 (five)
OIV-070	Mature leaf: anthocyanin coloration of the main veins on the upper side of the blade	3 (red until the first bifurcation)
OIV-072	Mature leaf: goffering of blade	3 (weak)
OIV-074	Mature leaf: profile	5 (rolled)
OIV-075	Mature leaf: blistering of upper side	3 (weak)
OIV-076	Mature leaf: shape of teeth	2 (both sides rectilinear)
OIV-079	Mature leaf: degree of petiole sinus opening	2 (opened)
OIV-080	Mature leaf: shape of base of petiole sinus	3 (V-shaped)
OIV-081-1	Mature leaf: presence of teeth in the petiole sinus	2 (occurrence of 1 or 2 teeth in the petiole sinus)
OIV-081-2	Mature leaf: petiole sinus limited by veins	3 (occurrence on both sides of petiole sinus)
OIV-083-2	Mature leaf: presence of teeth at the base of the upper leaf sinuses	1 (none)
OIV-084	Mature leaf: density of prostrate hairs between the main veins (lower side)	1 (none or very weak)
OIV-087	Mature leaf: density of erect hairs on the main veins (lower side)	1 (none or very weak)
OIV-151	Inflorescence: sex of flower	3 (hermaphrodite)
OIV-202	Cluster: length	5 (medium)
OIV-204	Cluster: density	5 (medium)
OIV-206	Cluster: length of peduncle	3 (short)
OIV-208	Cluster: shape	2 (narrow conical, broad conical)
OIV-209	Cluster: number of wings	2 (1-3 wings)
OIV-220	Berry: length	3 (short)
OIV-221	Berry: width	3 (small)
OIV-223	Berry: shape	2 (roundish)
OIV-225	Berry: colour of skin	6 (blue-black)
OIV-230	Berry: colour of flesh	1 (not coloured)
OIV-235	Berry: degree of firmness of flesh	5 (medium)
OIV-236	Berry: particular flavour	1 (none)
OIV-241	Berry: presence of seeds	3 (present)

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was performed. TPC, TA, VAN, and PA were determined using spectrophotometric analysis after the completion of alcoholic fermentation in October 2008.

HPLC analysis: The profile of different classes of polyphenols was determined in June 2009 using HPLC analysis. Determination of anthocyanidins and flavonols was performed according to MATTIVI *et al.* (2006), flavan-3-ols and proanthocyanidins according to FORTES GRIS *et al.* (2011a), stilbenes and tyrosol according to FORTES GRIS *et al.* (2011b), and hydroxycinna-mic and hydroxybenzoic acids according to VRHOVSEK *et al.* (2004).

The proanthocyanidin composition and mean degree of polymerization (mDP) were determined after acid catalysis in the presence of excess of phloroglucinol according to VRHOVSEK *et al.* (2004).

10. Descriptive sensory analysis

Sensory analysis was carried out after 60 days of bottle aging by a panel of 10 professionals (wine makers who are expert in the analysis of Croatian red wines): six women and four men. The evaluations were done at the Institute for Adriatic Crops and Karst Reclamation in Split, Croatia, using standard conditions for sensory evaluation. Wine samples (25 mL) were served in standard wine tasting glasses coded with random 3-digit numbers at a temperature between 15 and 17°C. Preliminary sensory evaluation of Dobričić aroma was done on two wine samples (two repetitions) to select the sensory characteristics and to obtain a list of descriptive aroma attributes. Attributes that were recognized by at least half of the judges were put on the list. During evaluation, the judges had reference solutions of 54 wine aromas included in the Le Nez du Vin Master kit (Brizard and Co, York, UK). In the second part of the evaluation, the judges were asked to rate from 0 to 9 the intensity of the attributes from the descriptive list of Dobričić. In this part of the evaluation, each judge evaluated two samples of Dobričić wine (two repetitions). Both evaluations were done on the same day, with a break in between. The panel supervisor did not take part in the evaluations.

RESULTS AND DISCUSSION

1. Ampelographic description

The distribution of Dobričić in commercial vineyards is currently very limited. During our inventory of native Croatian cultivars, we found it only in old vineyards on Šolta Island. In the past, Dobričić was widely cultivated in Central Dalmatia (BULIĆ 1949) but its cultivation declined, mainly due to modern development of urban environments and grower's preference for more productive cultivars such as Plavac Mali. Dobričić is the standard name for the cultivar and is also the most commonly used, although there are some local names still in use: Šoltanac, Muškić, Okručanac, and Slatinjanac.

The ampelographic description of the cluster, berry, leaf, and young shoot is shown in Table 1, according to the International grapevine descriptor list. The morphology of a typical Dobričić leaf was drawn using the main variables on which the identification was based (Fig. 2). The basic agronomic characteristics of Dobričić are listed in Table 2. The following is a short ampelographic description of Dobričić:

- Clusters: small to medium, 152.72 g weight, 16.48 cm length, 9.91 cm width; short conical shape; short peduncles; sometimes with one to three wings and loose to medium density.

- Berries: small, 0.92 g single berry weight; round shape; 176 berries per cluster; blue-black; flesh not coloured and medium firmness; with no particular flavour.

- Leaves: medium; pentagonal shape with five lobes; V-shaped petiolar sinus; rectilinear teeth; upper leaf sinuses slightly overlapping; lower leaf surface with very sparse prostrate hairs between the main veins.

- Shoot tips: open; weak density of prostrate hairs; young leaves are green.

- Yield and basic must composition: moderate yield, 1.84 kg/vine; moderate to low sugar concentrations, 19.6 ºBrix; low total titratable acidity, 4.7 g/L, pH 3.41.

The phenolic composition of grape seeds and skins is shown in Table 3. Dobričić is a highly coloured cultivar, which is a consequence of a high concentration of anthocyanins in the skin. The average total extractable anthocyanins in the skin was 1338 mg/kg. Comparison with previous data obtained using the same procedure (BUDIĆ-LETO *et al.,* 2008) showed that Dobričić has significantly more TA than the major red Croatian cultivar, Plavac Mali (879 mg/kg). Dobričić also has more VAN and PA in the grape skin.

The microsatellite profile of Dobričić at nine commonly used loci confirmed its unique genotype and perfectly matched those published by MALETIĆ *et al.* (2004) and the European *Vitis* Database (Table 4).

The high incidence of viruses in the field samples was revealed by ELISA tests. The most common infection was with GLRaV-3 (100 %) followed by GLRaV-1 (40 %), which coincides with results previously obtained on other cultivars in Croatian viticultural regions (KAROGLAN KONTIĆ *et al.,* 2009). The occurrence of GFLV was confirmed in 30 % of samples, and of GVA in 20 %. ArMV and GFkV were not detected. Relatively

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Table 2 - Agronomic characteristics in Dobričić evaluated at the original location on Šolta Island, Croatia, 2007-2010.

Characteristic	Mean	S.E.
Yield (kg/vine)	1.84	0.12
Cluster/vine	15	0.84
Cluster length (cm)	16.48	0.22
Cluster width (cm)	9.91	0.16
Cluster weight (g)	152.72	5.12
Berries/cluster	176	6.98
Berry weight (g)	0.92	0.03
Sugars (ºBrix)	19.6	0.34
Total titratable acidity (g/L)	4.7	0.35
pH	3.41	0.08

Mean, standard error of mean (SE); 4-year data

Table 3 - Average content of extractable polyphenols (mg/kg) in skin and seed extracts of Dobričić, 2007-2008.

	Skins	S.E.	Seeds	S.E.
Total phenols (mg/kg)[a]	2476	10	984	204
Vanillin indeks (mg/kg)[b]	1520	11	1091	353
Proanthocyanidins (mg/kg)[c]	2906	116	913	319
Total anthocyanins	1338	89	-	-

[a]equivalent of GAE; [b]equivalent of (+)-catechin; cequivalent of (+)-cyanidin
Standard error of mean (SE); 2-year data

Table 4 - The microsatellite profile of Dobričić at nine loci.

Locus	Allele size (bp)		Code*	
VVS2	145	151	N+22	N+28
VVMD5	228	228	N+6	N+6
VVMD7	247	249	N+16	N+18
VVMD27	179	179	N+4	N+4
VrZAG62	191	205	N+16	N+30
VrZAG79	237	259	N	N+22
VVMD32	253	265	N+17	N+29
VVMD25	243	245	N+4	N+6
VVMD28	251	261	N+34	N+42

*codes for alleles sizes proposed by GrapeGen06 project

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low yield and grape juice soluble solids in Dobričić could be partly due to high rates of viral infection in investigated vines and their known negative effects on production (WALTER and MARTELLI, 1998). However, the obtained results indicate the necessity to sanitize Dobričić vines through tissue culture and heat therapy. Given the grape sanitary status observed here, this likely had an impact on the morphological, productivity and wine composition results.

2. Wine composition

In the 2008 vintage, Dobričić produced wine with a moderate 12.2 % alcohol by volume; the reducing sugar concentration was below 5 g/L; the dry extract was 25.8 g/L; total acidity was 5.5 g/L and pH was very high (3.83).

The wine had more total phenols (2380 mg/L), total anthocyanins (995 mg/L), proanthocyanidins (2929 mg/L) and vanillin index (1911 mg/L) than the major Croatian red cultivar Plavac Mali (BUDIĆ-LETO *et al.,* 2008) and some other Croatian commercial wines (RASTIJA *et al.,* 2009).

a. Anthocyanin composition

Malvidin-3-glucoside was the dominant anthocyanin in the wine (69.3 %) (Table 5), a ratio similar to that in Merlot and Tannat wines (60 %) and Cabernet-Sauvignon wine (67 %) (GONZÁLEZ-NEVES *et al.*, 2001). In most wines from *Vitis vinifera* L. cultivars, malvidin-3-glucoside is the most abundant anthocyanin (MAZZA, 1995; KALLITHRAKA *et al.,* 2006). The relative amounts of each monoglucoside as a proportion of the total concentration of anthocyanins were as follows: petunidin-3-monoglucoside 10.1 %, delphinidin-3-glucoside 5.6 %, peonidin-3-glucoside 1.6 % and cyanidin-3-glucoside 0.3 %. The main acetyl and *p*-coumaroyl derivatives in wine were malvidin-3-glucoside-*p*-coumarate (5 %) and malvidin-3-glucoside acetate (3.6 %).

b. Free flavan-3-ol composition

The main flavan-3-ol monomers were catechin and epigallocatechin (Table 6). (+)-Catechin was present at a higher concentration than (-)-epicatechin. Cabernet-Sauvignon and Merlot wines (vintages from 1978 to 2005) also had (+)-catechin as the most abundant tannin monomer (CHIRA *et al.,* 2011). Among proanthocyanidin oligomers, proanthocyanidin B1 was the main dimer in wine. These results are consistent with published data on Cabernet Franc, Sangiovese, Syrah and Merlot wines (FORTES GRIS *et al.,* 2011a), but dimers B2 and B3 are the principal ones in Cabernet- Sauvignon wine (CHIRA *et al.,* 2011).

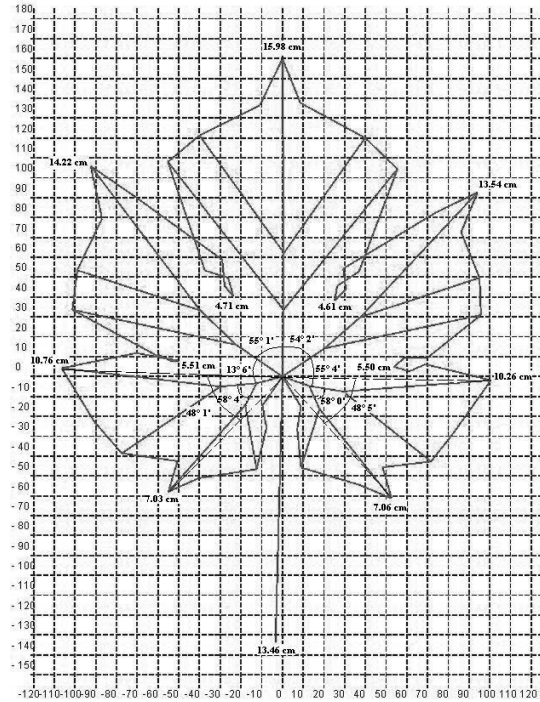


Figure 2 - Average Dobričić leaf, reconstructed following the method of SOLDAVINI *et al.* (2009).

c. Proanthocyanidin composition

Thioacidolysis showed that the terminal unit of proanthocyanidins consisted mainly of catechin and epigallocatechin, while epicatechin constituted the smallest percentage (Table 7). Upper and extension units of proanthocyanidins liberate catechin benzyl thioether, epicatechin benzyl thioether and gallocatechin benzyl thioether. Catechin was the main extension unit. The mean degree of polymerization (mDP) of 4.28 confirms that Dobričić wine is rich in monomers and small oligomers.

The mDP for Dobričić is similar to published data for Cabernet-Sauvignon and Merlot wines, in which mDP varies from 1.3 to 7.1 independently of cultivar and most likely depends on wine age (CHIRA *et al.,* 2011). In Bordeaux wines, mDP varies between 8.7 and 13.6 (DRINKINE *et al.,* 2007).

d. Flavonols

The main flavonols were myricetin (39.1 %) and quercetin (38.4 %), followed by laricitrin (7.7 %), syringetin (7.1 %), kaempferol (4.9 %) and isorhamnetin (2.8 %) (Fig. 3a). Concentrations of individual flavanols in Dobričić wine were higher than those reported in commercial Croatian wines from the same viticultural region (RASTIJA *et al.,* 2009). Previous research has shown that quercetin and

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Table 5 - Composition and concentration of anthocyanins and phenolic acids in Dobričić wine, 2008 vintage.

Compound	mg/L
Anthocyanins	
Delphinidin-3-monoglucoside	11.5
Cyanidin-3-monoglucoside	0.5
Petunidin-3-monoglucoside	20.8
Peonidin-3-monoglucoside	3.2
Malvidin-3-monoglucoside	143.1
Delphinidin-3-monoglucoside acetate	0.6
Cyanidin-3-monoglucoside acetate	0.1
Petunidin-3-monoglucoside acetate	0.7
Peonidin-3-monoglucoside acetate	0.6
Malvidin-3-monoglucoside acetate	7.5
Delphinidin-3-monoglucoside-*p*-coumarate	5
Cyanidin-3-monoglucoside-*p*-coumarate	0.6
Petunidin-3-monoglucoside-*p*-coumarate	0.8
Peonidin-3-monoglucoside-*p*-coumarate	1.4
Malvidin-3-monoglucoside-*p*-coumarate	10.3
Total monoglucosides	179.1
Total monoglucoside acetates	9.5
Total monoglucoside coumarates	18.1
Total free anthocyanins	206.7
Phenolic acids	
Gallic acid	19.1
Protocatechuic acid	6.4
Vanillic acid	1.4
Syringic acid	0.4
Total hydroxybenzoic acids	27.3
trans-Caftaric acid	61.1
cis-Coutaric acid	1.9
trans-Coutaric acid	7.9
Fertaric acid	4.6
Caffeic acid	3.9
p-Coumaric acid	1.8
Ferulic acid	0.6
Total hydroxycinnamic acids	81.8
Total phenolic acids	109.1
Tyrosol	97.5

myricetin are the main flavonols in red wines (MARKIS *et al.,* 2006), which is confirmed here.

e. Non-flavonoids

Among non-flavonoids, six hydroxycinnamic acids (*trans*-caftaric, *cis*- and *trans*-coutaric, fertaric, caffeic, *p*-coumaric and ferulic acid), four hydroxybenzoic acids (gallic, protocatechuic, vanillic and syringic) and tyrosol (Table 5), and four stilbenes (Fig. 3b) were identified.

f. Phenolic acids

The most abundant hydroxycinnamic acids were *trans*-caftaric (61.1 mg/L) and *trans*-coutaric (7.9 mg/L), while ferulic acid was the least abundant (0.6 mg/L). Among hydroxybenzoic acids, the highest concentrations were observed for gallic (19.1 mg/L) and protocatechuic (6.4 mg/L) acids, and the lowest for syringic acid (0.4 mg/L). The total concentration of hydroxycinnamic acids (81.8 mg/L) was higher than the total concentration of hydroxybenzoic acids (27.3 mg/L) (Table 5).

g. Stilbenes

The percentages of *trans*-resveratrol (22.07 % or 2.79 mg/L), *cis*-resveratrol (7.67 % or 0.97 mg/L), *trans*-piceid (25.71 % or 3.25 mg/L) and *cis*-piceid (44.54 % or 5.63 mg/L) are shown in Fig. 3b. The dominant monomer was *cis*-piceid. While it is the first report of *trans*- and *cis*-piceid in a Croatian wine, the concentration of resveratrol was within the determined values for other Croatian wines (RASTIJA *et al.* 2009).

3. Descriptive sensory analysis of Dobričić wine

Descriptive sensory analysis (DA) has been widely used to describe the sensory properties of wine and to define the aroma "fingerprint" of a varietal wine (CARLUCCI and MONTELEONE, 2008). We used DA to determine sensory attributes of wine produced from Dobričić harvested in 2008. Fifty-four sensory descriptors were chosen from the Wine Aroma Wheel (NOBLE *et al.,* 1987), of which seven (blackberry, black currant, cherry, dried prune, stemmy, bell pepper and mushroom) were agreed to define the aroma of Dobričić. A graphical representation of the typical sensory profile of Dobričić wine shows the mean values ± standard deviation of two repetitions for the sensory descriptors (Fig. 4). The most intense sensory attribute was blackberry (4.6), while the least intense was bell pepper (1.3). The dominating attributes were of berry fruits (blackberry, black currant, and cherry) but vegetative aroma (stemmy and bell pepper) was also noticed. The contrast between fruity and vegetative aromas has been observed in other red wines such as Cabernet- Sauvignon (HEYMANN and NOBLE, 1987), and it was recently demonstrated that

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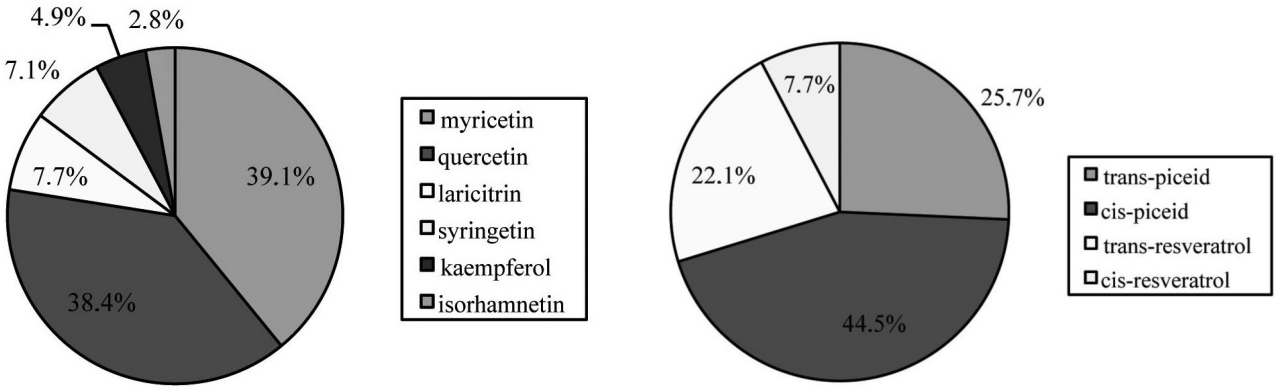


Figure 3 - Composition (percentage) of [A] flavonols and [B] stilbenes in Dobričić wine, 2008 vintage.

the vegetative character in wine could be masked by fruit aromas (HEIN *et al.,* 2009).

CONCLUSIONS

This study presents for the first time ampelographic and oenological characteristics of the Dobričić grapevine. Although Dobričić is currently grown in very small numbers in a very limited area, this study provides growers and winemakers with necessary information to bring Dobričić back into commercial production. In addition, Dobričić might be of interest for future breeding work. Further work, including creating healthy materials and experimenting with different viticultural practices and vinification techniques, would complete and expand these observations. Finally, this study demonstrates the importance of preserving genetic diversity in the European grapevine genetic pool.

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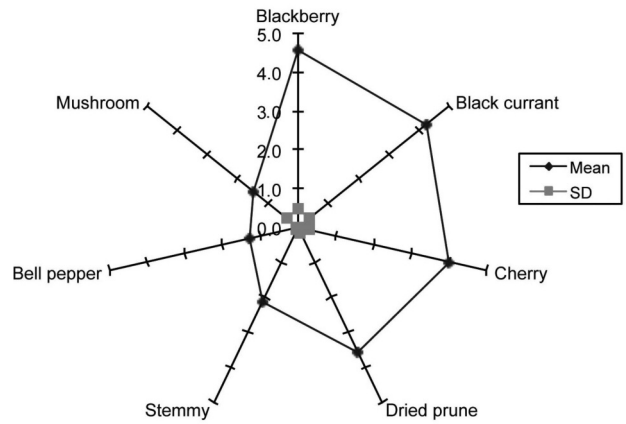


Figure 4 - Sensory profile of the intensity of the selected descriptors for the Dobričić monovarietal wine. Mean and standard deviation (SD) of sensory descriptors.

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Table 6 - Content of monomeric and dimeric flavan-3-ols in Dobričić wine, 2008 vintage.

Compound	mg/L
Catechin	32.09
Epicatechin	6.63
Gallocatechin	27.63
Epigallocatechin	31.98
Epicatechin gallate	n.d.
PA B1	57.24
PA B2	5.08
Total monomers	98.33
Total dimers	62.32

PA, proanthocyanidin

Table 7 - Structural characteristics and composition (Percent in Moles) of proanthocyanidins in Dobričić wine, 2008 vintage.

Unit	%
Terminal units	
Catechin	32.6
Epicatechin	6.7
Gallocatechin	28.1
Epigallocatechin	32.5
Epicatechin gallate	n.d.
Extension units	
Catechin	65.8
Epicatechin	5.8
Gallocatechin	15.9
Epigallocatechin	12.4
Total terminal units*	98.33
Total extension units*	286.31
mDP[A]	4.28

*Values in mg/L - [A]Mean degree of polymerization

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