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Anthocyanin profile of wild grape *Vitis vinifera* in the eastern Adriatic region

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ABSTRACT

Wild grapevine (*Vitis vinifera* L., subsp. *sylvestris*) was found recently along the rivers in the karst landscape of the eastern Adriatic region, which includes Croatia and Bosnia and Herzegovina. The wild grapevine is almost extinct and grows in a restricted habitat in Europe. The anthocyanin profile of red grape berries from naturally growing populations of female wild var. *sylvestris* were characterized in two consecutive years and compared to the domesticated cultivars 'Plavac mali', 'Merlot' and 'Xinomavro'. Five anthocyanidin-3-monoglucosides and their corresponding five 3-(6-*O*-acetyl) and five 3-(6-*O*-coumaryl) derivatives were identified and quantified using HPLC-DAD. Most wild grape samples had both acylated and non-acylated anthocyanin monoglucosides, although two individuals lacked acylated forms. DpGl were a greater percentage of the total in wild grape than in 'Plavac mali', 'Merlot' or 'Xinomavro'. Principal component analysis showed differentiation of *sylvestris* from *vinifera* by anthocyanin profile. The anthocyanin profiles of *sylvestris* genotypes from the eastern Adriatic region, here presented for the first time, suggest genetic diversity within *sylvestris* in anthocyanin synthesis.

1. Introduction

The anthocyanins determine the quality of red grape juice and wine and are an important trait for breeding programs. The colour of red grape berries depends on the concentrations and proportions of different anthocyanins accumulated in the skin. The proportions of individual anthocyanins are primarily determined by genotype and anthocyanin profiles can be used to discriminate red grapes and wines produced from different cultivars (Eder et al., 1994; Pomar et al., 2005; Nogales-Bueno et al., 2015; Pisano et al., 2015). The genetic control of anthocyanin biosynthesis is very complex, involving many different enzymes catalyzing each reaction (Boss et al., 1996).

The anthocyanin composition varies significantly among different species of the genus *Vitis* (Liang et al., 2008). Anthocyanins found in *Vitis vinifera* L. include malvidin, cyanidin, delphinidin, peonidin and petunidin 3-monoglycosides with their corresponding acetyl, *p*-coumaroyl and caffeoyl derivates, known as acylated anthocyanins. The absence of acylated anthocyanins is a very rare genetic character in *Vitis vinifera* L., found in the Pinot family of cultivars: 'Pinot noir' and its coloured mutants, 'Pinot gris', 'Pinot tete de negre' and 'Pinot meunier', produce no acylated forms. This genetic trait appears in several cultivars grown in the Rhine basin that are genetically closely related to 'Pinot noir', like 'Blauer Arbst' and 'Deckrtot' (Wulf and Nagel, 1978; Mattivi et al., 2006). Only two red cultivars from Southern Italy, 'Gaglioppo' and 'Tintilia', which produce berries with slightly coloured skins, lack acylated anthocyanins. Some grey and rosé cultivars like 'Muscat rouge de madèré', which are usually mutants of white cultivars, also lack acylated anthocyanins (Mattivi et al., 2006).

Non-*Vitis vinifera* grape species, widely used as rootstocks and sometimes cultivated for human consumption, contain 3,5-diglucosides and pelargonidin-derived anthocyanins. These are also found in hybrid cultivars like 'Concord', obtained by crossing *V. vinifera* and native American species like *V. labrusca* or *V. rupestris* (Wang et al., 2003; Liang et al., 2008).

The *Vitis* ssp. includes uncultivated wild species that represent a valuable source of genes for grapevine breeders focused not only on stable yields and high-quality grapes and wines, but also on good resistance of new cultivars to fungal diseases (Sun et al., 2016; Ruocco et al., 2017).

The color of grape was an important attribute during its domestication, directly affecting visual preference for fruits. Therefore, the anthocyanin profile of different varieties and species has been very intriguing for many grape researchers (Mattivi et al., 2006; Picariello et al., 2014). Different varieties with blue-black, deep dark (teinturier varieties) or distinctive red grape berry color have been developed. In grapevine breeding programs, *Vitis vinifera* was not always considered a good source of color, while some American *Vitis* species showed excellent potential for color enhancement. However, the role of American *Vitis* species in breeding programs has been limited due to their unpleasant impact on aroma (foxy aroma) (Sun et al., 2011; Narduzzi

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et al., 2015).

The wild *Vitis vinifera* L. subspecies *sylvestris* (Gmelin) Hegi is the sole wild grapevine existing in Europe and the ancestor of all cultivated *V. vinifera* varieties (Levadoux, 1956). *V. sylvestris* is an endangered or rare European plant and appears on the red list published by the International Union for Conservation of Nature (IUCN, 1997; Ocete et al., 2011, 2014).

Wild germplasm resources have not been widely explored, leaving the potential of these wild species to improve domesticated grape quality unknown (Revilla et al., 2010). Recently, the first research on the anthocyanin composition of wild grape accessions in a germplasm collection in Spain provided a characteristic fingerprint for several genotypes that was very similar to 'Pinot noir' and 'Gaglioppo' (Revilla et al., 2010; 2012). Some genotypes lacked acylated anthocyanins, although no domesticated Spanish red grape cultivar lacks acylated anthocyanins (Revilla et al., 2012).

The objectives of this study were: (i) to characterize the anthocyanins in wild *sylvestris* grape sampled from four naturally growing populations found recently in Croatia and Bosnia and Herzegovina and (ii) to evaluate whether the anthocyanin composition differs from that of cultivated grape. Individuals from the domesticated cultivars 'Merlot', 'Xinomavro' and 'Plavac mali', planted in the grapevine germplasm collection at the Institute for Adriatic Crops and Karst Reclamation in Split, (Dalmatia) Croatia, were selected for a reference. This is the first data on the anthocyanin composition of *V. sylvestris* populations growing in the eastern Adriatic region along the Mediterranean.

2. Materials and methods

2.1. Reagents and standards

Malvidin-3-*O*-glucoside chloride was obtained from Extrasynthese (Genay, France). LC–MS grade methanol and perchloric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Milli-Q water was used for the chromatography. All chemicals and reagents were AR or HPLC grade.

2.2. Plant material

Grapes from 11 previously identified (Zdunić et al., 2017) female individuals of *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi were collected from natural populations in Croatia and Bosnia and Herzegovina in 2014 and 2015 at their usual ripening time in this region, the end of September (Table 1). Because these grape samples were collected from vines growing on natural sites where ecology strongly affected the crop, it was not possible to collect grapes from all individuals in both years. Almost all clusters from wild individuals (~1.000 g/vine) were collected. Three replicate samples of 100 berries from different cluster positions were randomly selected.

Cultivated grape berries of *Vitis vinifera* L. cvs. 'Plavac mali', 'Merlot' and 'Xinomavro' were collected from the germplasm collection at the Institute for Adriatic Crops and Karst Reclamation in Croatia at technological maturity in both years. Three replicate samples of 100 berries from different vines and positions were collected from each cultivar. The grape berries were taken to the laboratory immediately after harvest and peeled. The skins were frozen at −70 °C and lyophilized.

2.3. Extraction of anthocyanins from grape skin

The freeze-dried skin samples were ground into powder using an electric grinder. Five hundred mg powder was extracted with 10 mL acidified methanol (methanol/water/perchloric acid 80/15/5, v/v/v) in a cooled ultrasonic bath based on a modified method (Will and Dietrich, 2013). Up to three successive extractions were performed if colour remained in the powder. The liquid extract and powdered grape skin were separated by centrifugation at 4000 rpm for 15 min. For each sample, the extracts were combined in a volumetric flask and brought up to 50 mL with extraction solution. The extracts were analyzed within three to four hours.

2.4. HPLC-DAD analysis of anthocyanins

The analysis of anthocyanins used a Varian HPLC system (Varian, Inc., Harbour City, CA, USA) consisting of a Star 9010 pump, a Rheodyne 7125 syringe-loading sample injector, a 500-LC module for a column oven, and a ProStar 330 Photodiode Array Detector and were controlled using the Star Chromatography workstation, version 5. Separation was carried out using a Kinetex C18 core-shell column (150 × 4.6 mm) filled with five-µm particles and furnished with a SecurityGuard ULTRA Cartridge UHPLC C18 for 4.6 mm ID column (Phenomenex, Torrance, CA, USA), both thermostated at 35 °C. Anthocyanins were identified and quantified as described in other publications (Vanzo et al., 2008; Fredotović et al., 2017).

The samples and standards were filtered through a 0.45 µm membrane syringe filter prior to analysis. Anthocyanins were identified by retention time and the UV-DAD spectra of each peak and quantified at 520 nm using a calibration curve made with malvidin-3-*O*-glucoside chloride (Extrasynthese, Genay, France). The resulting concentrations were expressed as mg/100 g dry weight and converted to percentages.

2.5. Statistical analysis

Statistically significant differences between the anthocyanin fingerprint data of wild and cultivated samples were determined using one-way analysis of variance (ANOVA) in each year using STATISTICA 10 software (StatSoft Inc., Tulsa, OK, USA). Differences tested by

Table 1
Wild and cultivatedgrapevines in Croatia and Bosnia and Herzegovina and their year(s) of sampling.

Genotype	Species	Site	Country of collecting/cultivation	Year
G1	*V. vinifera* subsp. *sylvestris*	Gizdavac	Croatia	2014, 2015
IJK 90	*V. vinifera* subsp. *sylvestris*	Paklenica	Croatia	2014
IJK 92	*V. vinifera* subsp. *sylvestris*	Paklenica	Croatia	2014
IJK 96	*V. vinifera* subsp. *sylvestris*	Paklenica	Croatia	2014
PAK1	*V. vinifera* subsp. *sylvestris*	Paklenica	Croatia	2015
NE14	*V. vinifera* subsp. *sylvestris*	Neretva	Bosnia and Herzegovina	2014
NE17	*V. vinifera* subsp. *sylvestris*	Neretva	Bosnia and Herzegovina	2014, 2015
NE18	*V. vinifera* subsp. *sylvestris*	Neretva	Bosnia and Herzegovina	2014
NE19	*V. vinifera* subsp. *sylvestris*	Neretva	Bosnia and Herzegovina	2015
IM3	*V. vinifera* subsp. *sylvestris*	Modro jezero	Croatia	2015
IM10	*V. vinifera* subsp. *sylvestris*	Modro jezero	Croatia	2015
'Plavac mali'	*V. vinifera* subs. *sativa*	IAC collection	Croatia	2014, 2015
'Xinomavro'	*V. vinifera* subs. *sativa*	IAC collection	Croatia	2014, 2015
'Merlot'	*V. vinifera* subs. *sativa*	IAC collection	Croatia	2014, 2015

Table 2
Anthocyanin profiles of *sylvestris* and cultivated genotypes ('Plavac mali', 'Xinomavro' and 'Merlot'), expressed as percentage as the % total anthocyanin concentration ± standard deviation.

Genotype	Year	DpGl	CyGl	PtGl	PnGl	MvGl	Sum Ac	Sum Qum
G1	2014	23.9 ± 0.91^{a}	8.9 ± 0.45^{e}	17.7 ± 0.38^{a}	8.6 ± 0.32^{b}	29.0 ± 1.02^{d}	5.6 ± 1.37^{b}	6.3 ± 0.42^{c}
	2015	26.7 ± 2.25^{B}	9.4 ± 0.91^{E}	18.3 ± 0.98^{F}	7.3 ± 0.19^{B}	20.5 ± 2.23^{E}	12.8 ± 1.70^{A}	4.3 ± 0.50^{A}
IJK 90	2014	24.7 ± 0 .35^{a}	8.8 ± 0.15^{e}	13.3 ± 0.22^{c}	12.9 ± 0.12^{e}	25.2 ± 0.21^{c}	6.3 ± 1.06^{b}	8.7 ± 0.30^{a}
	2015	–	–	–	–	–	–	–
IJK 92	2014	28.4 ± 0.88^{e}	23.5 ± 0.31^{f}	12.5 ± 0.11^{bc}	20.9 ± 0.49^{h}	14.8 ± 0.18^{b}	0.0 ± 0.00^{a}	0.0 ± 0.00^{b}
	2015	–	–	–	–	–	–	–
IJK 96	2014	16.8 ± 0.03^{d}	4.3 ± 0.02^{ac}	12.0 ± 0.19 ^{b}	15.7 ± 0.21^{f}	51.2 ± 0.01^{g}	0.0 ± 0.00^{a}	0.0 ± 0.00^{b}
	2015	–	–	–	–	–	–	–
PAK1	2014	–	–	–	–	–	–	–
	2015	25.4 ± 0.30^{B}	4.0 ± 0.01^{D}	14.4 ± 0.12 ^{A}	6.3 ± 0.26^{A}	32.1 ± 0.08^{B}	4.2 ± 0.53^{BC}	13.6 ± 0.27^{F}
NE14	2014	23.8 ± 0.33^{a}	2.9 ± 0.00^{a}	18.6 ± 0.23 ^{a}	3.1 ± 0.07^{a}	33.7 ± 0.52^{e}	9.7 ± 1.01^{d}	8.1 ± 0,00^{a}
	2015	–	–	–	–	–	–	–
NE17	2014	23.4 ± 3.72^{a}	5.6 ± 2.94^{cd}	16.9 ± 2.29 ^{d}	5.6 ± 2,59^{c}	31.6 ± 1.78^{a}	5.1 ± 1.28^{bc}	11.8 ± 3.19^{d}
	2015	19.2 ± 0.25^{B}	1.3 ± 0.03^{BC}	16.8 ± 0.58 ^{E}	1.9 ± 0.11^{C}	37.7 ± 0.64^{G}	13.5 ± 1.39^{A}	9.5 ± 0.01^{C}
NE18	2014	23.7 ± 0.48^{a}	2.6 ± 0.08^{ab}	18.1 ± 0.32 ^{ad}	2.9 ± 0.08^{a}	31.6 ± 0.32^{a}	12.3 ± 1.11^{e}	8.8 ± 0.17^{a}
	2015	–	–	–	–	–	–	–
NE19	2014	–	–	–	–	–	–	–
	2015	19.3 ± 0.65^{A}	11.5 ± 0.54^{F}	14.5 ± 0.09^{A}	15.0 ± 0.67^{E}	25.7 ± 0.75^{F}	6.5 ± 0.17^{B}	7.5 ± 0.32^{b}
IM3	2014	–	–	–	–	–	–	–
	2015	19.8 ± 1.40^{A}	3.0 ± 0.22^{A}	15.4 ± 0.95^{A}	7.0 ± 0.76^{AB}	29.0 ± 1.82^{AB}	14.7 ± 2.63^{A}	11.1 ± 0.72^{D}
IM10	2014	–	–	–	–	–	–	–
	2015	17.8 ± 0.11^{A}	3.2 ± 0.71^{AD}	14.6 ± 0.37^{A}	7.2 ± 0.01^{AB}	31.1 ± 2.08^{AB}	14.1 ± 1.11^{A}	12.1 ± 0.22^{E}
Plavac	2014	12.8 ± 0.00^{b}	2.7 ± 0.12^{ab}	10.1 ± 0.05^{g}	7.6 ± 0.02^{b}	45.5 ± 0.97^{f}	1.0 ± 1.13^{a}	20.3 ± 0.30^{f}
	2015	13.8 ± 0.57^{E}	2.8 ± 0.18^{A}	11.4 ± 0.31^{D}	7.0 ± 0.08^{AB}	43.8 ± 0.33^{CD}	2.1 ± 0.45^{C}	19.1 ± 0.57^{H}
Xinomavro	2014	3.4 ± 0.26^{c}	1.3 ± 0.15^{b}	4.1 ± 0.12^{e}	10.0 ± 0.42^{d}	55.1 ± 0.85^{h}	3.4 ± 0.82^{c}	22.7 ± 0.38^{g}
	2015	5.1 ± 0.00^{C}	0.7 ± 0.0^{B}	5.6 ± 0.00^{B}	4.5 ± 0.00^{D}	46.6 ± 0.00^{D}	7.8 ± 0.00^{B}	29.8 ± 0.00^{I}
Merlot	2014	10.8 ± 0.15^{b}	6.8 ± 0.00^{d}	8.5 ± 0.01^{f}	18.3 ± 0.08^{g}	31.4 ± 0.61^{a}	9.7 ± 0.88^{d}	14.5 ± 0.05^{e}
	2015	9.9 ± 0.32^{D}	1.8 ± 0.12^{C}	8.7 ± 0.10^{C}	6.4 ± 0.37^{A}	42.0 ± 1.14^{C}	13.8 ± 0.91^{A}	17.3 ± 0.46^{G}

DpGl: delphinidin 3 glucoside; CyGl: cyanidin 3-glucoside; PtGl: petunidin 3-glucoside; PnGl: peonidin 3-glucoside; MvGl: malvidin 3-glucoside; Sum Ac: sum of acetylated derivatives of anthocyanins; Sum Qum: sum of *p*-coumarylated derivatives of anthocyanins; and -: not sampled. Values are reported as LSD by Fisher's test at $p \leq 0.05$.
Small letters within columns denote significant differences in 2014 and capital letters within columns denote significant differences in 2015.

Fisher's least significant difference (LSD) were considered significant at $p \leq 0.05$. Principal component analysis (PCA) as a multivariate analytical tool was performed by XLSTAT version (2018).2.50918 (Addinsoft, Paris, France).

3. Results and disscusion

3.1. Anthocyanin fingerprint of sylvestris

The anthocyanin compositions of wild grape from four locations in the eastern Adriatic region were identified and quantified in 2014 and 2015. Anthocyanins are divided into five basic group based on their anthocyanidin: delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn) and malvidin (Mv). We found 15 different anthocyanins in grape samples from four locations: the 3-monoglucosides of delphinidin (DpGl), cyanidin (CyGl), petunidin (PtGl), peonidin (PnGl), and malvidin (MvGl); their acetylated derivatives (DpGlAc, CyGlAc, PtGlAc, PnGlAc, and MvGlAc) and their *p*-coumarylated derivatives (DpGlCm, CyGlCm, PtGlCm, PnGlCm, and MvGlCm). Acylated anthocyanin concentrations are presented as a percentage of the total concentration of anthocyanins (Table 2).

As is typical in reverse-phase chromatography, the compounds were eluated in order of polarity, which is also the order of presentation in Fig. 1.

The chromatograms of G1, IJK 92 and IJK 96 and 'Merlot' at 520 nm are presented as typical (Fig. 1). In wild grape genotype G1 (Fig. 1A), all 15 anthocyanin peaks were identified. The first five peaks represent the non-acylated anthocyanins DpGl, CyGl, PtGl, PnGl, and MvGl, while the later peaks represent the ten acylated anthocyanins. The samples IJK 92 and IJK 96, found in the Paklenica population, had no acylated anthocyanins. The chromatogram of genotypes IJK 92 (Fig. 1B) and IJK 96 (Fig. 1C) had only five peaks corresponding to the 3-monoglucosides of DpGl, CyGl, PtGl, PnGl and MvGl. Similar results have been reported previously (Revilla et al., 2012).

The concentrations of DpGl, CyGl, PtGl, PnGl, MvGl and their corresponding acylated forms varied significantly among genotypes ($p \leq 0.05$). Two different groups of genotypes were evident. Group one included genotypes that produced only monoglucosides (IJK 92 and IJK 96), while the second group (populations Gizdavac, Modro jezero and Neretva) produced monoglucosides and acylated derivatives of monoglucosides. Absence of acylated anthocyanins was recently found in 23 female *sylvestris* among 126 accessions preserved in the Germplasm Bank in Spain (Revilla et al., 2012). There were significant differences in the anthocyanin profiles of two genotypes, IJK 92 and IJK 96, both of which did not produce acylated anthocyanins. The most abundant anthocyanin in IJK 96 was MvGl (51.2%) and in IJK 92, DpGl (28.4%). The specific anthocyanin fingerprint of those few *sylvestris* individuals with a predominance of DpGl was associated with methyltransferase activity, which adds a methyl group onto the 5´ position of delphinidin to produce malvidin (Boss et al., 1996). MvGl was the primary anthocyanin in most genotypes in 2014 and 2015.

In 2014, the most MvGl was found in NE14 (33.7%), which differed significantly from other *sylvestris*. In 2015, NE17 had the most MvGl (37.7%), while the proportion of MvGl in other *sylvestris* ranged from

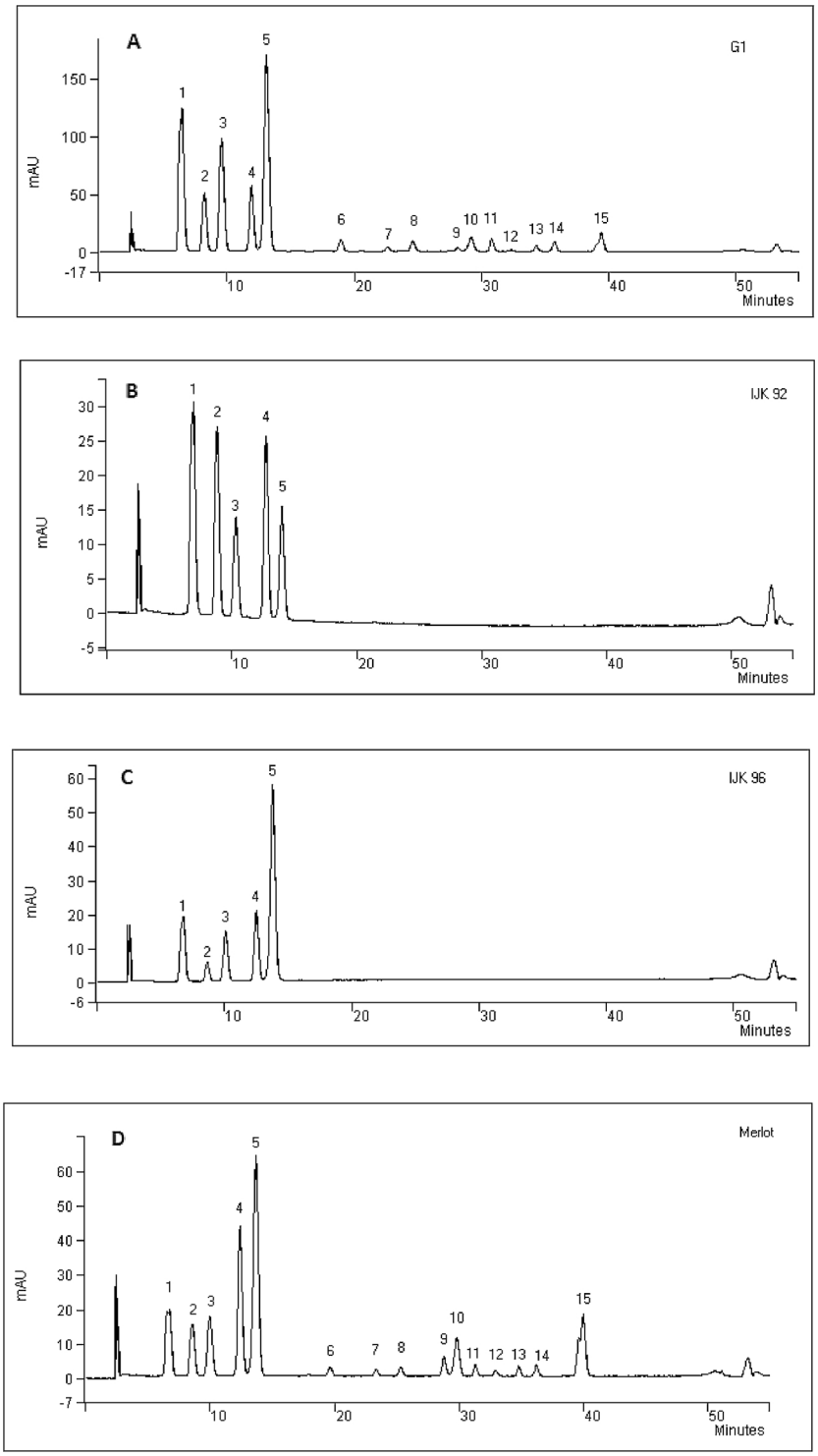

Fig. 1. Chromatograms at 520 nm for grape samples G1 (A), IJK 92 (B), IJK 96 (C) and 'Merlot' (D). Peaks were identified as follows: 1) delphinidin 3-monoglucoside, 2) cyanidin 3-monoglucoside, 3) petunidin 3-monoglucoside, 4) peonidin 3-monoglucoside, 5) malvidin 3-monoglucoside, 6) delphinidin 3-monoglucoside-acetate, 7) cyanidin 3-monoglucoside-acetate, 8) petunidin 3-monoglucoside-acetate, 9) peonidin 3-monoglucoside-acetate, 10) malvidin 3-monoglucoside-acetate, 11) delphinidin 3-monoglucoside-*p*-coumarate, 12) cyanidin 3-monoglucoside-*p*-coumarate, 13) petunidin 3-monoglucoside-*p*-coumarate, 14) peonidin 3-monoglucoside-*p*-coumarate and 15) malvidin 3-monoglucoside-*p*-coumarate.

20.5% in G1 to 31.1% in IM10.

The predominate anthocyanins in genotype IJK 92 were DpGl (28.4%) and CyGl (23.5%), which were significantly more abundant than in the other genotypes. DpGl was the second-most abundant anthocyanin in genotypes G1, IJK 90, PAK 1, NE14, NE17 and NE18, accounting for over 20% of total anthocyanins. The most abundant DpGl was found in IJK 90 and G1, at 24.7% and 26.7% of the total anthocyanins, respectively. There were no significant differences in DpGl among IJK 90, G1, NE14, NE17 and NE18 in 2014 or among G1, PAK1 and NE17 in 2015. PtGl was the third-most abundant anthocyanin produced by wild grapevines. The concentrations of PtGl exceeded 15% of the total anthocyanins in G1, NE14, NE17, NE18 and IM3. Anthocyanins in *sylvestris* are monoglycosidic, while in American wild species of genus *Vitis,* anthocyanins also occur as diglucosides (Liang et al., 2012). Anthocyanin profiles could be used as reliable chemotaxonomic criteria to discriminate between *vinifera* and non-*vinifera* genotypes (Picariello et al., 2014).

3.2. Comparison of anthocyanin profiles of wild and cultivated grapes

The 3-glucosides of delphinidin (DpGl), cyanidin (CyGl), petunidin (PtGl), peonidin (PnGl) and malvidin (MvGl), the 6-acetyl esters of the

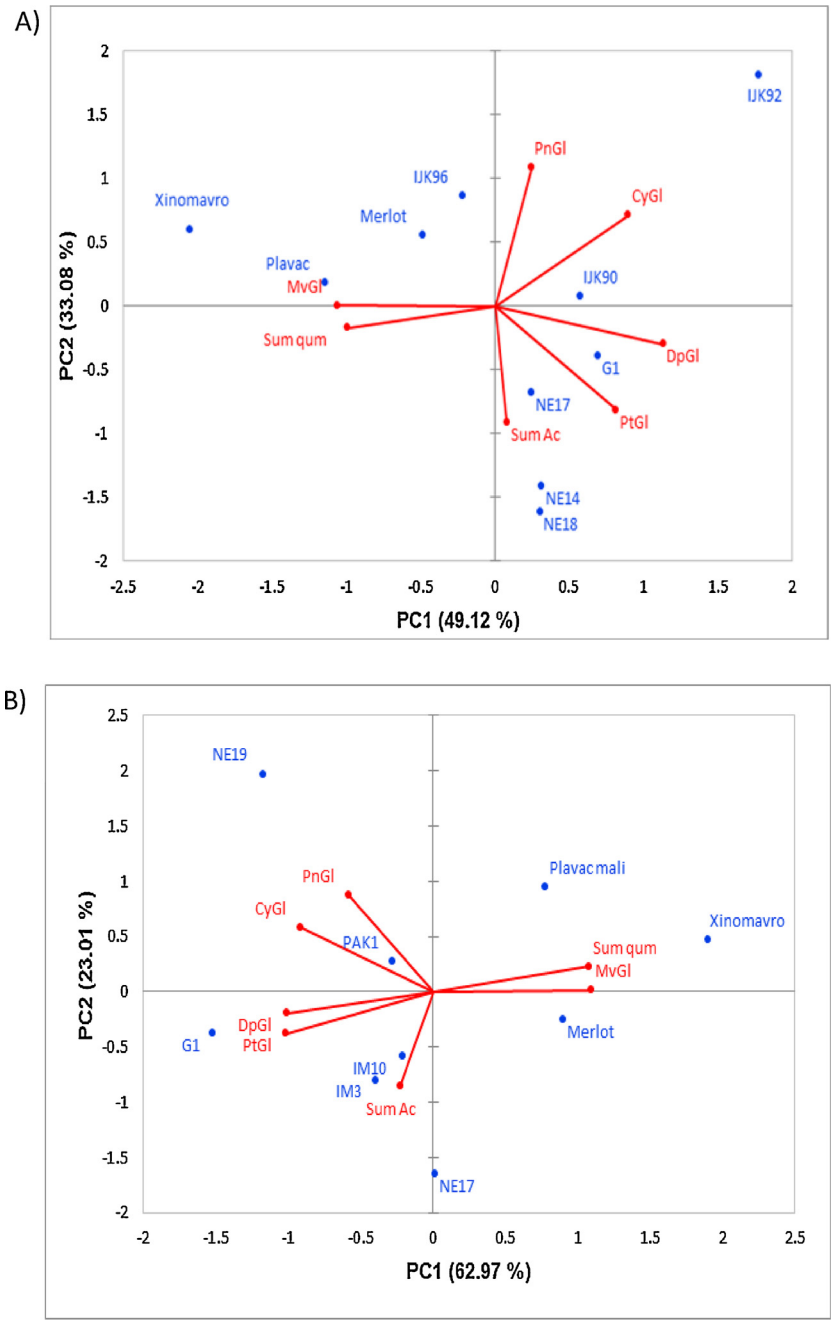

Fig. 2. Biplots of the applied principal component analysis (PCA) of 10 samples from the 2014 vintage (Picture A) and nine samples from the 2015 vintage (Picture B) on the plane defined by principal components PC1 and PC2. (*V. sylvestris* samples: Gizdavac: G1, Paklenica: IJK 90, IJK 92, IJK 96, PAK1; Modro Jezero: IM3, IM10; Neretva: NE14, NE17, NE18, NE19; V. *sativa* samples: 'Merlot'; 'Plavac mali'; 'Xinomavro') Abbreviations: DpGl: delphinidin 3-monoglucoside; CyGl: cyanidin 3-monoglucoside; PtGl: petunidin 3-monoglucoside; PnGl: peonidin 3-monoglucoside; MvGl: malvidin 3-monoglucoside; Sum Ac: sum of acetylated derivatives of anthocyanins; and Sum Qum: sum of *p*-coumarylated derivatives of anthocyanins.

3-glucosides of delphinidin, (DpGlAc), cyanidin (CyGlAc), petunidin (PtGlAc), peonidin (PnGlAc) and malvidin (MvGlAc), and the 6-*p*-coumaryl esters of the 3-glucosides of delphinidin (DpGlCm), cyanidin (CyGlCm), petunidin (PtGlCm), peonidin (PnGlCm) and malvidin (MvGlCm) were identified and quantified in *Vitis vinifera* cvs. 'Merlot' and 'Xinommavro' and 'Plavac mali', a native Croatian cultivar. MvGl was the major anthocyanin in cultivated grapes, constituting 31% of anthocyanins in 'Merlot' in 2014 and 42% in 2015, 45% in 'Plavac' mali in both years, and 47% in 'Xinommavro' in 2014 and 55% in 2015. These proportions were greater than those found in wild grapevine genotypes. The anthocyanin composition for cultivated grapes reported here is in good agreement with previous studies (Mattivi et al., 2006; Lorrain et al., 2011; Ćurko et al., 2014). These previous results showed predominance of MvGl in the anthocyanin composition of domesticated grapevines: 41% of the anthocyanin in 'Plavac mali' from Croatia (Ćurko et al., 2014) and 44% in 'Merlot' from Bourdoux (Lorrain et al., 2011). A similar anthocyanin pattern was found in 'Merlot' from Italy (Mattivi et al., 2006). The primary anthocyanin in 'Merlot' was MvGl, constituting 35% of total anthocyanins, following by DpGl at 9% and PnGl at 8%. The sum of acetylated derivatives was 22% and the sum of *p*-coumarylated derivatives, 15%. Some grape cultivars used to produce premium red wines, such as 'Merlot', contain remarkable quantities of acylated anthocyanins (Mazza, 1995).

Results from this study showed that berries from a few *sylvestris* genotypes like IM3, IM10, NE14, NE17, and NE18 also contained some acetylated and *p*-coumarylated anthocyanin derivatives, highlighting their possible role in winemaking. Their enological potential and possible use in breeding should be investigated in future studies.

DpGl was more abundant in wild grape samples than in 'Merlot', 'Xinommavro' and 'Plavac mali'. Greater abundance of Dp and Cy derivates was found in wild *Vitis* species than in cultivated ones (Liang et al., 2012). However, the composition of acylated anthocyanins (presented in Table 2 as the sum of *p*-coumarylated and acetylated derivates of five anthocanins) were found at lower percentages in

berries from wild grape genotypes than in cultivated 'Merlot', 'Xinommavro' and 'Plavac mali'.

3.3. PCA analysis

The data were processed using principal component analysis (PCA) to group samples according to their anthocyanin profile. The PCA was performed separately for samples in each vintage. The results were presented as biplots to simplify the multivariate observation analysis. In a projection of seven anthocyanin variables that defined the latent PC factors 1 and 2, these first two PCs accounted for 49.12% and 33.08% of the variance in 2014 and 62.97% and 23.01% of the variance in 2015, respectively (Fig. 2).

In 2014, DpGl and CyGl had large positive loadings on PC1, while MvGl and Sum Qum had large negative loadings on PC2. In 2015, in contrast, MvGl and Sum Qum had positive loadings on PC1, while DpGl and PtGl had large negative loadings on PC2.

PCA clearly separated samples by taxonomic identity (subspecies *sativa* or *sylvestris*) in both years. In 2014 (Fig. 2A), cultivars 'Plavac mali', 'Merlot' and 'Xinomavro' separated together into the upper left part of the diagram (negative part of PC2). It is interesting to note the positioning of IJK 92 and IJK 96 from Paklenica due to their lack of acylated anthocyanins. While IJK 92 was isolated from the other *sylvestris* and *sativa* samples, IJK 96 grouped very close to the cultivars.

In 2015 (Fig. 2B), the grouping of samples was very similar, although there was no consistent sample set of individuals. The cultivated samples 'Plavac mali', 'Merlot' and 'Xinomavro' grouped distinct from *sylvestris* samples. *Sylvestris* samples did not group very close to each other, highlighting their variable anthocyanin composition. PCA confirmed that the anthocyanin composition was related to the taxonomic identity of samples.

4. Conclusions

The first data on the anthocyanin profile of female *Vitis vinifera* L. subspecies *sylvestris* (Gmelin) Hegi individuals, originated from a recently discovered population in Croatia and Bosnia and Herzegovina, is presented and compared to the anthocyanin composition in cultivated varieties 'Plavac mali', 'Merlot' and 'Xinomavro'. Cultivated and wild grapevine genotypes could be differentiated using anthocyanin profiles. Among 11 wild genotypes, only two (IJK 92 and IJK 96) lacked acylated anthocyanins, while only one wild genotype (IJK 92) had a predominance of delphinidin 3-glucosides. This work improves our knowledge of the chemical composition of *sylvestris* grapes that could be beneficial if used in genetic breeding programs and modern wine-making production.

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