

## ORIGINAL PAPER

Comparison of anthocyanins present in grapes of *Vitis vinifera* L. varieties and interspecific hybrids grown in the Czech Republic<sup>a</sup>Josef Balík, <sup>b</sup>Michal Kumšta, <sup>c</sup>Otakar Rop\*<sup>a</sup>Department of Post-Harvest Technology of Horticultural Products, <sup>b</sup>Department of Viticulture and Oenology, Faculty of Horticulture, Mendel University in Brno, Valtická 337, 691 44 Lednice, Czech Republic<sup>c</sup>Department of Food Technology and Microbiology, Faculty of Technology, Tomas Bata University in Zlín, Náměstí TGM 275, 762 72 Zlín, Czech Republic<sup>†</sup>

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Anthocyanins present in the grapes of nine grapevine (*Vitis vinifera* L.) varieties and ten interspecific hybrids were compared. Total anthocyanin levels were determined in fresh grapes and the values ranged from 0.50 g kg<sup>-1</sup> to 4.99 g kg<sup>-1</sup>. A total of twenty-two different anthocyanins were identified. Malvidin 3-glucoside was the most abundant anthocyanin, with concentrations ranging from 30 % to 64 % of the totals observed. The ratio of acetylated to coumaroylated malvidin and peonidin derivatives ranged from 0.09 (variety: Regent) to 1.34 (variety: Cerason), depending on the variety. Diglycosylated anthocyanins were not found in particular interspecific hybrids such as the teinturier XIV 26-56, Laurot, Merlan, and Nativa.

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**Keywords:** grape, *Vitis vinifera* L., interspecific hybrids, teinturier, anthocyanins**Introduction**

Anthocyanins are the most common group of water-soluble plant pigments. Due to the fact that their molecules contain a carbon skeleton of C6—C3—C6, they are classified among naturally-occurring phenols as flavonoids (Timberlake, 1980). Basically, they are heteroglycosides consisting of a saccharide component and an aglycon (anthocyanidin). In grapes of *V. vinifera* L. there are five coloured aglycons commonly occurring, namely cyanidin, peonidin, delphinidin, petunidin, and malvidin (Mazza & Francis, 1995). Another aglycon, pelargonidin, has been reported by Zhao et al. (2010) in grapes of *V. amurensis* and its hybrids.

Anthocyanin molecules are always glycosylated either in the C-3-*O* position (monoglycosides) or additionally in the C-5-*O* position (diglycosides), and to

date only glucose and one unidentified pentose have been found in the anthocyanins which occur in grapes (Mazzuca et al., 2005).

The saccharide component of an anthocyanin is often acylated with some organic acids. In grapes, the anthocyanins are most frequently acylated with *p*-coumaric and acetic acids, but also occasionally with caffeic, pyruvic, and ferulic acids (Revilla et al., 2001; He et al., 2010). García-Beneytez et al. (2003) also identified small amounts of the *cis*-isomers occurring alongside the predominant *trans*-isomers of anthocyanins acylated with *p*-coumaric acid. Alcalde-Eon et al. (2006) were the first to identify anthocyanins acylated with lactic acid in wine made from the variety Tempranillo.

In the course of ripening, anthocyanins accumulate in the berry skins (and, in the case of teinturier varieties, also in the pulp), and are responsible for

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the various shades of the resulting colour, which can range from light red to dark blue and almost black (González-SanJosé & Diez, 1992; Brar et al., 2008). Katalinić et al. (2010) observed total anthocyanins in red grapes of *V. vinifera* L. grown in Croatia. Anthocyanin contents were variety-dependent and ranged from 158 mg kg<sup>-1</sup> to 1848 mg kg<sup>-1</sup> of fresh grape berry.

The relative abundance of the individual anthocyanins has a variety-specific character, and the anthocyanin profile of any given variety remains broadly the same regardless of where it is grown. Different territories and variations between growing seasons result mainly in changes in the overall levels of anthocyanins (Mori et al., 2005; Downey et al., 2006; González-Neves et al., 2007; Revilla et al., 2009).

Dark-blue grapes of the majority of *V. vinifera* L. varieties typically contain acylated monoglucosylated anthocyanins, of which malvidin 3-glucoside (formerly oenin) is the most common (Mazza & Francis, 1995; García-Beneytez et al., 2002). Acetyl anthocyanins were more abundant in *V. vinifera* grapes than in other species of grapes (Zhu et al., 2012). Diglucosylated anthocyanins are more typical of grapes of other members of the *Vitis* genus (e.g. *V. riparia*, *V. labrusca*, *V. rupestris*, *V. rotundifolia*, etc.) and the interspecific hybridisation of American grapevine species with the European grapevine (*V. vinifera* L.) means that this dominant diglucosylated trait is then also found in French-American hybrids (Goldy et al., 1986; Hebrero et al., 1989). However, in attempting to increase the resistance of vines to certain diseases, a number of new interspecific hybrids have been selected by backcrossing them with European varieties of *V. vinifera* L. (Kozma, 2000), and the diglucosylated anthocyanins are therefore absent from the grapes of some interspecific hybrids (Lamikanra, 1989; Mazzuca et al., 2005).

This study sought to compare the overall levels of anthocyanins and the anthocyanin profiles of grapes from a range of *V. vinifera* L. varieties and interspecific hybrids grown in the Czech Republic.

## Experimental

### Material and methods

Total anthocyanin levels were determined in the grapes of nine varieties of grapevine (*V. vinifera* L.): André (An), Blaufrankisch (Fr), Blauer Portugieser (MP), Cabernet Moravia (CM), Dornfelder (Do), the teinturier Neronet, Pinot Noir (RM), Saint Laurent (Sv), and Zweigeltrebe (Zw) (Ne), as well as in ten interspecific hybrids: Cerason (Ce), the teinturier Golubok (Go), the teinturier XIV 18-32 (Mo.1), the teinturier XIV 26-56 (Mo.2), 81-7-4 (Mo.3), Kaberon (Ka), Laurot (La), Merlan (Mer), Nativa (Na), and Regent (Re).

The analyses were performed over a period of six consecutive years (2004–2009) using grapes sourced from the wine-growing sub-region of Mikulov in the wine-growing region of Moravia (EU wine-growing zone B), where the average annual temperature is 9.42 °C and the average precipitation is 510 mm. The vineyard co-ordinates are 48°8' N and 16°6'–16°9' E and their soils are classified as calcareous clays and sands.

The grapes were sampled at a stage of harvest maturity typical of the Czech Republic, and sugar levels fluctuated in the range of 17.5–21.0 °Brix depending on the year; these are common values in maturity in northerly regions. The grapes (ten bunches per cultivar per year) were stored at a temperature of –18 °C for a maximum period of three weeks.

All chemical reagents used were obtained from Fluka (purchased from Sigma–Aldrich, Prague, Czech Republic).

The anthocyanins were extracted using a modification of the method described by Drdak et al. (1991). A total of 10–15 g of whole berries (skin and pulp) for each bunch was extracted by discontinuous homogenisation in a mixer in three steps. Each step of the extraction was performed with 30 mL of acidified methanol (0.1 % hydrochloric acid in 100 % methanol) for 15 min and centrifugation for 3 min at 3000 min<sup>-1</sup>. Subsequently, the three extracts were combined and the final extract was made up to the volume of 100 mL with acidified methanol (0.1 % hydrochloric acid in 100 % methanol). The anthocyanins in this centrifuged extract were then analysed.

The total content of anthocyanins was estimated by a spectrophotometric method published by Fuleki and Francis (1968); the method is based on measuring the differences in absorbance values of the anthocyanin extract obtained in buffers with pH 1 (0.2 mol L<sup>-1</sup> of potassium chloride and 0.2 mol L<sup>-1</sup> of hydrochloric acid (in the ratio of 25 : 67)) and pH 4.5 (1.0 mol L<sup>-1</sup> of sodium acetate and 1.0 mol L<sup>-1</sup> of hydrochloric acid and deionised water (in the ratio of 100 : 60 : 90)) at 520 nm (spectrophotometer Helios  $\beta$ /Unicam, Pye Unicam, Cambridge, UK). The concentration of total anthocyanins was expressed on the basis of the content of malvidin 3-glucoside using values of molar absorptivity (28000 L mol<sup>-1</sup> cm<sup>-1</sup>) and molecular mass (562.5 g mol<sup>-1</sup>) (Fernández-López et al., 1992). The values thus obtained were converted to express the contents of anthocyanins in fresh grapes as g kg<sup>-1</sup>.

The extracts from whole berries were separated and identified using the Shimadzu binary high pressure system LC-10A coupled with DAD detection SPD-M10Avp (Shimadzu Europa GmbH, Duisburg, Germany) at 520 nm: 20  $\mu$ L was injected into Alltech Alltima C18 3  $\mu$ m (3 mm  $\times$  150 mm + pre-column 3 mm  $\times$  7.5 mm (Shimadzu Europa GmbH, Duisburg, Germany)); separation temperature was 60 °C. Gradi-

**Table 1.** Names and origins of *Vitis vinifera* L. varieties and interspecific hybrids

Acronym	Variety (Crossbreeding)	Crossing/Origin	Species/Teinturier
An	André	Blaufrankisch × Saint Laurent/Czech Republic	<i>Vitis vinifera</i> L./no
Ce	Cerason (Mi 5-100)	Merlan × (Blaufrankisch × Saint Laurent)/Czech Republic	interspecific/no
CM	Cabernet Moravia	Zweigeltrebe × Cabernet Franc/Czech Republic	<i>Vitis vinifera</i> L./no
Do	Dornfelder	Helfensteiner × Heroldrebe/Germany	<i>Vitis vinifera</i> L./no
Fr	Blaufrankisch	–	<i>Vitis vinifera</i> L./no
Go	Golubok	Severnyj × (Alicante Bouschet × Cabernet Sauvignon)/Ukraine	interspecific/yes
Ka	Kaberon (Mi 5-26)	Merlan × (Blaufrankisch × Saint Laurent)/Czech Republic	interspecific/no
La	Laurot (Mi 5-106)	Merlan × (Blaufrankisch × Saint Laurent)/Czech Republic	interspecific/no
Mer	Merlan (XIV 11-57)	Merlot × Seibel 13 666'/Czech Republic	interspecific/no
Mo.1	XIV 18-32	Gamay fréaux × (Saperavi × Seyve Villard 23 657')/Moldova	interspecific/yes
Mo.2	XIV 26-56	Seyve Villard 18 315' × (Ceaus rozovyyj × Mičurinec)/Moldova	interspecific/yes
Mo.3	81-7-4	<i>Vitis amurensis</i> × Tramin/Moldova	interspecific/no
MP	Blauer Portugieser	–	<i>Vitis vinifera</i> L./no
Na	Nativa	(Blaufrankisch × Saint Laurent) × Merlan/Czech Republic	interspecific/no
Ne	Neronet	(Saint Laurent × Blauer Portugieser) × (Alicante Bouschet × Cabernet Sauvignon)/Czech Republic	<i>Vitis vinifera</i> L./yes
Re	Regent	(Sylvaner × Müller Thurgau) × Chambourcin/Germany	interspecific/no
RM	Pinot noir	–	<i>Vitis vinifera</i> L./no
Sv	Saint Laurent	–	<i>Vitis vinifera</i> L./no
Zw	Zweigeltrebe	Saint Laurent × Blaufrankisch/Austria	<i>Vitis vinifera</i> L./no

ent elution began with a flow-rate of 0.6 mL min<sup>-1</sup> for mobile phase A (15 mM of perchloric acid). Mobile phase B (5 mM of perchloric acid, 10 % of methanol, 50 % of acetonitrile) was used as follows: 0 min, 2 % of B; 20 min, 26 % of B; 30 min, 45 % of B; 35 min, 70 % of B; 37 min, 100 % of B; 38 min, 0 % of B; 40 min, 2 % of B; 45 min 2 % of B.

Malvidin 3,5-diglucoside (peak 7) and malvidin 3-glucoside (peak 10) were identified on the basis of standards (purchased from Sigma–Aldrich, Prague, Czech Republic). Other anthocyanins separated on the C18 column were identified on the basis of the retention times published by Burns et al. (2002) and García-Beneytez et al. (2003). The order of anthocyanins separated in a reverse-phase chromatographic column is constant under the same conditions of chromatographic analysis (Manns & Mansfield, 2012).

The reproducibility of the method was verified by spiking one sample from each series with malvidin 3-glucoside and malvidin 3,5-diglucoside. Recovery of the standards was in the range of 98–102 %. Retention times ranged from 21.95 min to 22.08 min for malvidin 3,5-diglucoside and from 26.23 min to 26.41 min for malvidin 3-glucoside.

Each chemical parameter was measured in triplicate. The data obtained were analysed statistically by the analysis of variance (ANOVA), Tukey's multiple range test for comparison of means and cluster analysis using the statistical package Unistat, v. 6.1.

## Results and discussion

### Total content of anthocyanins

The content of anthocyanins was measured in the

grapes of nine grapevine (*V. vinifera* L.) varieties traditionally grown in the Czech Republic (in the total area of 5000 hectares) and also ten interspecific hybrids, which are currently cultivated over a relatively small area of 10 hectares (Table 1).

The highest level of pigments was found in the interspecific hybrids Mo.1 and Mo.2 (4.80 g kg<sup>-1</sup> and 4.99 g kg<sup>-1</sup> of fresh grapes, respectively). *Nativa* also contains a high amount of total anthocyanins (4.42 g kg<sup>-1</sup>), though this interspecific hybrid is not the teinturier. Average levels of pigments above 2.00 g kg<sup>-1</sup> of fresh grapes were recorded only in Laurot, Cerason, and Dornfelder (Table 2).

The following varieties and interspecific hybrids constituted another statistical group, without any statistically significant differences in the contents of total anthocyanins in whole bunches of fresh grapes: Merlan, Zweigeltrebe, Mo.3, Kaberon, Saint Laurent, Regent, and Cabernet Moravia (1.27–1.85 g kg<sup>-1</sup>). Again, with reference to whole bunches of grapes, relatively low levels of pigments were found in the following varieties: Pinot Noir, Blauer Portugieser, and Blaufrankisch (0.50–0.86 g kg<sup>-1</sup>) (Table 3). Mazza and Francis (1995) found similar low levels of total anthocyanins in grapes of the Pinot Noir variety (0.33 g kg<sup>-1</sup>), while grapes of the teinturier Alicante-Bouschet contained up to as much as 5.2 g kg<sup>-1</sup> of these pigments. Orak (2007) found contents of total anthocyanins in the range of 0.04–0.99 g kg<sup>-1</sup> of fresh berry mass in the varieties observed. In general, De Rosso et al. (2012) noted that the overall levels of anthocyanins in selected hybrid grape varieties ranged from 0.48 g kg<sup>-1</sup> to 5.29 g kg<sup>-1</sup>. Lamikanra (1989) found much higher concentrations of total an-

**Table 2.** Total anthocyanins content in fresh grapes of *Vitis vinifera* L. varieties and interspecific hybrids for six vintages

Acronym <sup>a</sup>	<i>Vitis vinifera</i> L.	Teinturier	Content/(g kg <sup>-1</sup> )	
			Mean <sup>b</sup>	SD <sup>c</sup>
An	yes	no	1.10 <sup>abc</sup>	0.25
Ce	no	no	2.22 <sup>de</sup>	0.27
CM	yes	no	1.85 <sup>def</sup>	0.32
Do	yes	no	2.29 <sup>e</sup>	0.29
Fr	yes	no	0.86 <sup>ag</sup>	0.18
Go	no	yes	3.00 <sup>h</sup>	0.21
Ka	no	no	1.65 <sup>cdf</sup>	0.28
La	no	no	2.20 <sup>de</sup>	0.22
Mer	no	no	1.27 <sup>abcf</sup>	0.35
Mo.1	no	yes	4.99 <sup>i</sup>	0.45
Mo.2	no	yes	4.80 <sup>i</sup>	0.50
Mo.3	no	no	1.64 <sup>cdf</sup>	0.25
MP	yes	no	0.71 <sup>ag</sup>	0.15
Na	no	no	4.42 <sup>ij</sup>	0.59
Ne	yes	yes	3.93 <sup>j</sup>	0.27
Re	no	no	1.81 <sup>def</sup>	0.31
RM	yes	no	0.50 <sup>g</sup>	0.17
Sv	yes	no	1.78 <sup>def</sup>	0.35
Zw	yes	no	1.32 <sup>bef</sup>	0.41

a) For individual acronym, see Table 1; b) different superscripts in each column indicate significant differences in the mean at  $P < 0.05$ ; c) standard deviation.

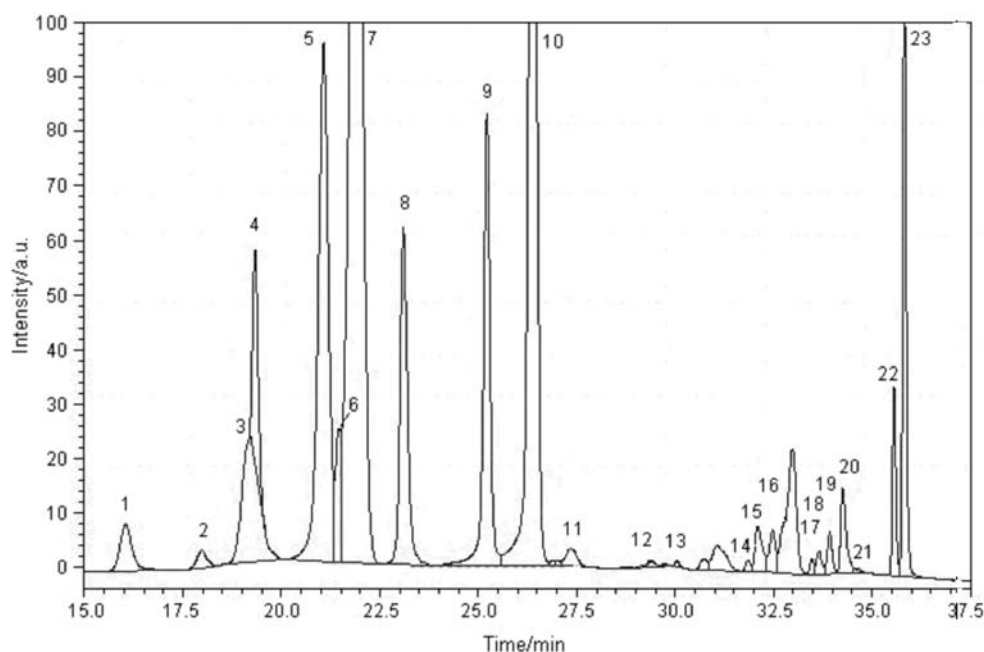
thocyanins in *V. rotundifolia* (0.12–10.38 g kg<sup>-1</sup> of fresh berry mass).

### Anthocyanin profiles

Anthocyanin extracts from grapes were analysed using HPLC-DAD detection (Fig. 1). In all, twenty-three different anthocyanins were separated, of which twenty-two were identified, i.e. about 97.5 % of the total peak area (Table 3). In the grapes analysed, the most abundant individual anthocyanin pigments were malvidin 3-glucoside (39 %), malvidin 3-(6''-*O*-coumaroyl)glucoside (9 %), delphinidin 3-glucoside (8 %), peonidin 3-glucoside (8 %), and petunidin 3-glucoside (8 %). Together, these five compounds made up 72 % of total anthocyanins. Anthocyanins with cyanidin occurred in lower amounts in the grapes of *V. vinifera* L. varieties. Cyanidin levels exceeding 10 % of the total were seen only in the interspecific hybrids Cerason and Mo.3.

Anthocyanidins glycosylated only with glucose were identified in all cases. In respect of the one unidentified anthocyanin with the retention time of 34.7 min, this could be an optical isomer of anthocyanin acylated with *p*-coumaric acid, as described by García-Beneytez et al. (2003).

Compared with the other samples, very low lev-



**Fig. 1.** HPLC-DAD chromatogram of anthocyanins in berry skins of interspecific hybrid Golubok. Peaks identification: 1 – delphinidin 3,5-diglucoside; 2 – cyanidin 3,5-diglucoside; 3 – petunidin 3,5-diglucoside; 4 – delphinidin 3-glucoside; 5 – peonidin 3,5-diglucoside; 6 – cyanidin 3-glucoside; 7 – malvidin 3,5-diglucoside; 8 – petunidin 3-glucoside; 9 – peonidin 3-glucoside; 10 – malvidin 3-glucoside; 11 – delphinidin 3-(6''-*O*-acetyl)glucoside; 12 – cyanidin 3-(6''-*O*-acetyl)glucoside; 13 – petunidin 3-(6''-*O*-acetyl)glucoside; 14 – peonidin 3-(6''-*O*-acetyl)glucoside; 15 – malvidin 3-(6''-*O*-acetyl)glucoside; 16 – delphinidin 3-(6''-*O*-coumaroyl)glucoside; 17 – peonidin 3-(6''-*O*-caffeoyl)glucoside; 18 – cyanidin 3-(6''-*O*-coumaroyl)glucoside; 19 – malvidin 3-(6''-*O*-caffeoyl)glucoside; 20 – petunidin 3-(6''-*O*-coumaroyl)glucoside; 21 – unidentified; 22 – peonidin 3-(6''-*O*-coumaroyl)glucoside; 23 – malvidin 3-(6''-*O*-coumaroyl)glucoside.

**Table 3.** Anthocyanin composition (%) in grapes of *Vitis vinifera* L. varieties and interspecific hybrids identified by HPLC–DAD (only total average characteristic of each variety is given)

$t_R$ / min	Antho- cyanin	An <sup>a</sup>	Ce	CM <sup>a</sup>	Do <sup>a</sup>	Fr <sup>a</sup>	Go	Ka	La	Mer	Mo.1	Mo.2	Mo.3	MP <sup>a</sup>	Na	Ne <sup>a</sup>	Re	RM <sup>a</sup>	Sv <sup>a</sup>	Zw <sup>a</sup>
16.0	Dp-3,5diGl	nd	nd	nd	nd	nd	1.27	nd	nd	nd	5.39	nd	4.75	nd	nd	nd	0.26	nd	nd	nd
18.0	Cy-3,5diGl	nd	nd	nd	nd	nd	0.46	nd	nd	nd	2.31	nd	5.74	nd	nd	nd	tr	nd	nd	nd
19.0	Pt-3,5diGl	nd	nd	nd	nd	nd	2.38	nd	nd	nd	8.65	nd	4.29	nd	nd	nd	0.94	nd	nd	nd
19.4	Dp-3Gl	3.93	20.26	5.29	8.53	2.49	5.26	3.90	11.36	11.76	12.75	11.17	18.82	0.36	9.21	8.69	3.37	4.61	10.98	5.49
21.0	Pn-3,5diGl	nd	nd	nd	nd	nd	12.31	0.10	nd	nd	8.95	nd	9.59	nd	nd	nd	2.72	nd	nd	nd
21.6	Cy-3Gl	0.74	7.85	0.47	3.32	0.65	1.52	1.39	1.31	0.76	4.06	5.07	13.81	tr	1.72	3.35	0.73	2.00	1.92	0.56
22.0	Mv-3,5diGl	nd	nd	nd	nd	nd	29.04	0.23	nd	nd	20.91	nd	28.20	nd	nd	nd	21.36	nd	nd	nd
23.2	Pt-3Gl	4.81	12.80	5.97	10.07	3.48	5.46	7.66	11.34	11.03	6.23	13.14	5.49	0.50	10.35	9.26	5.26	6.21	9.58	5.89
25.4	Pn-3Gl	7.15	2.81	5.89	13.38	11.36	6.74	9.93	2.60	1.37	3.47	14.81	1.95	1.07	7.16	17.00	1.64	20.89	7.99	5.39
26.3	Mv-3Gl	49.67	21.61	47.52	40.20	60.90	21.28	60.46	37.95	37.52	5.49	39.74	7.03	52.61	45.77	30.09	19.33	64.38	41.37	48.13
27.5	Dp-3GlAc	0.27	5.20	0.47	1.08	tr	0.41	tr	2.28	2.22	0.75	0.36	nd	0.20	0.24	1.59	0.44	nd	1.39	0.72
29.2	Cy-3GlAc	0.17	2.02	0.14	0.41	tr	tr	tr	0.36	0.22	1.88	0.17	tr	0.25	tr	0.58	0.70	tr	0.37	0.18
30.0	Pt-3GlAc	0.50	4.15	0.76	1.63	tr	tr	0.19	3.20	2.73	0.30	0.47	nd	0.35	0.37	1.92	0.22	tr	1.63	0.91
31.9	Pn-3GlAc	0.94	0.73	0.83	1.66	0.19	0.12	0.45	0.65	0.45	0.36	0.60	tr	0.67	0.52	2.65	0.38	tr	1.50	0.58
32.6	Mv-3GlAc	7.54	7.31	7.57	6.33	1.56	0.58	2.11	9.85	8.49	0.45	1.61	nd	10.82	2.04	6.93	1.04	0.12	7.98	9.40
32.1	Dp-3GlCm	0.84	3.88	1.20	1.00	0.29	0.74	0.34	2.68	3.34	4.01	1.33	tr	0.38	1.99	1.19	3.22	tr	1.58	1.42
33.4	Pn-3GlCf	0.10	tr	0.10	0.51	0.12	0.13	0.54	tr	tr	0.11	0.27	tr	0.13	0.40	0.64	0.12	tr	tr	tr
33.7	Cy-3GlCm	0.19	1.50	0.14	0.39	tr	0.26	0.15	0.41	0.34	1.10	0.87	nd	tr	0.45	0.66	0.45	tr	0.38	0.13
33.9	Mv-3GlCf	1.15	0.21	0.94	1.80	0.68	0.44	2.47	0.26	0.77	0.45	0.82	nd	6.95	2.46	1.34	1.81	tr	0.26	0.41
34.3	Pt-3GlCm	1.02	2.47	1.47	1.17	0.45	1.04	0.79	2.76	3.25	1.80	1.57	nd	0.95	2.25	1.35	4.60	tr	1.37	1.53
34.7	Un	0.44	tr	0.56	0.13	0.54	tr	tr	0.20	0.34	tr	0.12	nd	0.43	0.13	0.10	0.16	tr	0.11	0.32
35.6	Pn-3GlCm	2.54	0.70	2.38	1.71	2.23	1.40	1.35	1.00	0.71	0.85	2.12	tr	0.54	1.86	3.72	1.22	0.17	2.00	1.60
35.8	Mv-3GlCm	17.08	5.31	17.74	5.87	13.97	4.58	7.19	10.87	13.88	2.07	5.07	0.10	16.08	12.26	8.24	14.46	0.50	8.52	16.73
$\sum$ (%)		99.04	98.81	99.44	99.19	98.90	95.42	99.25	99.08	99.18	92.34	99.31	99.77	92.29	99.18	99.30	84.43	98.83	98.93	99.39

a) *Vitis vinifera* L. varieties (for varieties/hybrids acronyms, see Table 1);  $t_R$  – retention time; Un – unidentified; nd – not detected; tr – traces; Dp – delphinidin; Cy – cyanidin; Pt – petunidin; Pn – peonidin; Mv – malvidin; Gl – glucoside; diGl – diglucoside; Ac – acetyl; Cf – caffeoyl; Cm – coumaroyl.

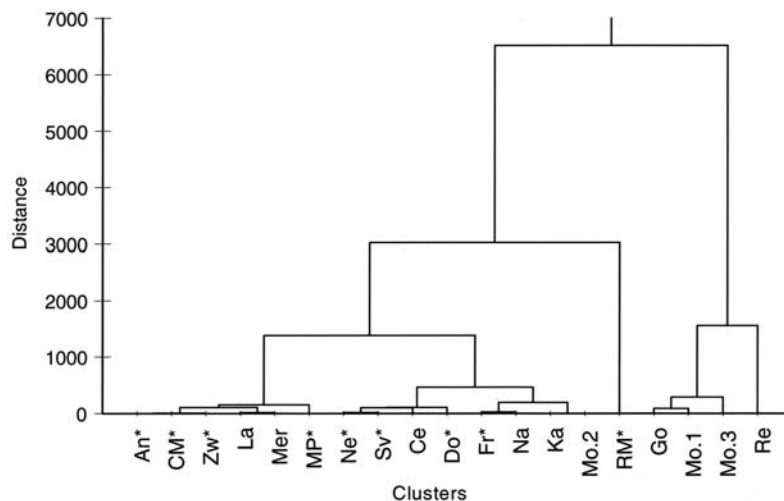
**Table 4.** Proportions of main anthocyanin groups (%) in grapes of *Vitis vinifera* L. varieties and interspecific hybrids

Acronym	ANTC-Gl <sup>a</sup>	ANTC-diGl <sup>b</sup>	Ac(Mv and Pn) <sup>c</sup>	Cm(Mv and Pn) <sup>d</sup>	$\sum$ (Ac and Cm) <sup>e</sup>	Ac/Cm <sup>f</sup>
An <sup>g</sup>	66.3	0.0	8.5	19.6	28.1	0.43
Ce	65.3	0.0	8.0	6.0	14.1	1.34
CM <sup>g</sup>	65.1	0.0	8.4	20.1	28.5	0.42
Do <sup>g</sup>	75.5	0.0	8.0	7.6	15.6	1.05
Fr <sup>g</sup>	78.9	0.0	1.7	16.2	17.9	0.11
Go	40.3	45.5	0.7	6.0	6.7	0.12
Ka	83.3	0.3	2.6	8.5	11.1	0.30
La	64.6	0.0	10.5	11.9	22.4	0.88
Mer	62.4	0.0	8.9	14.6	23.5	0.61
Mo.1	32.0	46.2	0.8	2.9	3.7	0.28
Mo.2	83.9	0.0	2.2	7.2	9.4	0.31
Mo.3	47.1	52.6	0.0	0.1	0.1	0.18
MP <sup>g</sup>	54.6	0.0	11.5	16.6	28.1	0.69
Na	74.2	0.0	2.6	14.1	16.7	0.18
Ne <sup>g</sup>	68.4	0.0	9.6	12.0	21.5	0.80
Re	30.3	25.4	1.4	15.7	17.1	0.09
RM <sup>g</sup>	98.1	0.0	0.1	0.7	0.8	0.20
Sv <sup>g</sup>	71.8	0.0	9.5	10.5	20.0	0.90
Zw <sup>g</sup>	65.5	0.0	10.0	18.3	28.3	0.54

a) Non-acetylated anthocyanin glucosides; b) anthocyanin diglucosides; c) acetylated malvidin and peonidin; d) *p*-coumaroylated malvidin and peonidin; e) sum of acetylated and *p*-coumaroylated; f) ratio of acetylated and *p*-coumaroylated; g) *Vitis vinifera* L. varieties/hybrids (for acronyms, see Table 1).

els of malvidin 3-glucoside were found in interspecific hybrids Mo.1 and Mo.3 (5.49 % and 7.03 %, respectively). In *V. vinifera* L., average levels of individual

pigments were as follows: 49 % of malvidin 3-glucoside, 12 % of malvidin 3-(6''-O-coumaroyl)glucoside, 10 % of peonidin 3-glucoside, 6 % of petunidin 3-glucoside,



**Fig. 2.** Dendrogram of varieties and hybrids based on their anthocyanin composition (Hierarchical Cluster Analysis); *Vitis vinifera* L. varieties (for varieties/hybrids acronyms, see Table 1) are identified by asterisk.

and 6 % of delphinidin 3-glucoside. In total, this represents 83 % of total content of anthocyanins. Malvidin 3-glucoside occurred in all three of its acylated forms, i.e. not only with coumaric and acetic but also with caffeic acid. Other types of anthocyanidins exhibited significantly lower proportions of acylated anthocyanins (Table 4).

#### ***Evaluation of acetylated and diglucosylated anthocyanins***

In comparing the levels of acetylated and coumaroylated monoglucosylated anthocyanins, the grapes of the majority of *V. vinifera* L. varieties and interspecific hybrids consistently exhibited a preponderance of the coumaroylated form (Table 4). This is typical of *V. vinifera* L. varieties and can be used to verify the authenticity of certain red wines (Burns et al., 2002). Higher levels of the acetylated form were found only in Dornfelder and Cerason, and approximately equal amounts were observed in Saint Laurent and the interspecific hybrid Laurot. Furthermore, markedly low levels of acetylated anthocyanins were found in Blaufrankisch, which is a typical red wine variety of the Central European wine-growing regions (Czech Republic, Slovakia, Hungary, Austria (Papoušková et al., 2011)). No acylated anthocyanins were identified among the diglucosylated anthocyanins. García-Beneytez et al. (2002) reported a predominance of coumaroylated anthocyanins in grapes of the Monastrell and Garnacha varieties. On the other hand, acetylated anthocyanins predominated in grapes of Cabernet Sauvignon and Merlot varieties.

Cluster analysis (Fig. 2) grouped varieties and interspecific hybrids with the highest amounts of acylated monoglucosylated anthocyanins (André, Cabernet Moravia, Zweigeltrebe, Laurot, Merlan, and Blauer Portugieser) on the left. Pinot Noir occupies

a unique position in this dendrogram because its grapes contained only trace concentrations of acylated monoglucosylated anthocyanins. Clusters of interspecific hybrids containing diglucosylated anthocyanins (Golubok, Kaberon, Mo.1, Mo.3, and Regent) are grouped on the far right.

As far as the other interspecific hybrids (Mo.2, Laurot, Merlan, and Nativa) are concerned, no diglucosylated anthocyanins were found in their grapes (Table 4). This observation confirms the opinion that the absence of diglucosides cannot be considered sufficient evidence of a variety's origins. Following the interspecific hybridisation of American species with the European grapevine (*V. vinifera* L.), the dominant diglucosylated trait is seen in grapes of the original French-American hybrids; however, after backcrossing with European grapevine varieties, this trait may then be completely absent.

#### **Conclusions**

Anthocyanins were measured in the grapes of nine common grapevine (*V. vinifera* L.) varieties and ten interspecific hybrids grown in the Czech Republic. Total anthocyanin content and anthocyanin profiles were identified. The results obtained in most samples have not yet been described. Given that the anthocyanin profile of grapes is typical of each *V. vinifera* variety, the results may serve as an important biochemical aspect for utilisation of berries of particular varieties. However, the most significant contribution is that an analysis of the anthocyanin profile presented in this paper using nine anthocyanins from grapes (i.e. glucoside derivatives of malvidin, delphinidin, cyanidin, petunidin, peonidin as well as acetylated and coumaroylated malvidin 3-glucoside and peonidin 3-glucoside) can be used to verify the authenticity of red wines.

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