Three Nebbiolo clone anthocyanin profile as affected by environmental conditions

Influence du terroir sur les composés anthocyaniques de trois clones de Nebbiolo

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Abstract: *Vitis vinifera* 'Nebbiolo' cultivar is a 3'-subsituted anthocyanin prevalent wine variety. It is grown in North-West Italy for the production of high quality ageing wines. In the present work berry skin anthocyanin amounts and profiles of the clones CVT 308, CVT 423 and CVT 142 were studied in 2004 and in 2005 in four environmentally different locations of North-West Italy: Donnas (steep mountain area), Monforte (hilly area, with a pH of 8.1), Vezza (hilly area, with a pH of 8.2) and Lessona (plain area, with a pH of 4.8). The interaction cultivation area vs climatic condition of the year was studied in relation to the clone anthocyanin contents and profiles. Differences in the anthocyanin amounts and profile were kept among sites and in both years and they allowed the discrimination among sites. CVT 308 and CVT 423 showed some analogies in three sites only in 2005, while the CVT 142 anthocyanin composition was similar to the one of clone CVT 423 in Donnas and of clone CVT 308 both in Donnas and in Lessona, but only in 2005. Grapes from Vezza accumulated more sugars and less anthocyanins showing higher percentages of malvidin-3-O-glucoside and of total acylated derivatives respect to the other locations.

Key words: *Vitis vinifera*, environment, climate, anthocyanin amount, anthocyanin percentage

Introduction

Nebbiolo grape is an important red cultivar grown in North Italy for the production of high quality wines. It shows a wide range of within variety variability, presenting numerous biotypes that often show differential morphological and attitudinal characteristics. Nebbiolo wine anthocyanin amount is rather low due to the predominant concentration of di-substituted anthocyanin in grapes (peonidin-3-O-glucoside, in particular), notably less stable than other anthocyanins during the wine making process. Thus, it is very important for this variety to assess which technical and/or environmental factors can influence the total anthocyanin amounts and the anthocyanin profile.

As reported by many authors, light, temperature and the interactive effects of light and temperature highly influence the phenolic accumulation in berry skins (Roubelakis-Angelakis and Kliewer, 1986; Keller and Hrazdina 1998; Haselgrove *et al.* , 2000; Bergqvist *et al.* , 2001; Spayd, *et al.*, 2002). Other factors, particularly those linked to soil (Yokotszuka *et al.*, 1999), water availability (Esteban *et al.*, 2001; Deloire *et al.*, 2001; Kennedy *et al.*, 2002, Peterlunger *et al.*, 2002), *terroir* (Vivas de Gaulejac *et al.*, 2001, Van Leeuwen *et al.*, 2004), or virus infection in the vine (Guidoni *et al.*, 1997) can modify the berry anthocyanin amount and profile (Keller and Hrazdina 1998, Haselgrove *et al.*, 2000, Vivas de Gaulejac *et al.*, 2001; Guidoni *et al.*, 2002, Downey *et al.*, 2004) at harvest.

The aim of the present work was to evaluate the influence exerted by the cultivation area on the anthocyanin synthesis of three Nebbiolo clone berry skins, putting in evidence the different environment suitability, i.e. the *terroir* effects. The year effect on the vine genotypic features was evaluated by repeating the trial in two years and in different geographical and pedological areas.

Materials and methods

The trial was carried out in 2004 and 2005 in four vineyards located in distinct geographical areas of North-West Italy (table 1). The examined areas are highly suitable for the production of Nebbiolo based wines undergone to more or less prolonged ageing periods. The studied vineyard cultural practices, typical of the

different areas of the trial, can be considered as *terroir* factors: the Donnas vineyard differed from the other vineyards being placed on terraces with vines trained to the pergola trellis system, double Guyot pruned, and showing the lowest vine density (table 2).
The studied clone CVT 142 selected in Langhe area (Piedmont region) belonged to the Lampia biotype, whereas the clones CVT 308 and CVT 423, selected in Aosta Valley Region, belonged to the Picotener biotype. At commercial harvest 150 berries were collected from the upper, the middle and the bottom part of different cluster sides. Berry skins of three replicates of ten berries each were separated from the pulp; anthocyanin were extracted by a hydro-alcoholic buffer solution, pH 3.2, containing 2 g L^{-1} of $Na_2S_2O_5$. The extract was analysed by HPLC and the individual anthocyanins were identified comparing the retention time of each chromatographic peak with available data in literature (Di Stefano and Maggiorotto, 1995). The amount of individual anthocyanins (mg kg^{-1} of berry fresh weight) was expressed as malvidin-3-O-glucoside using malvidin-3-O-glucoside chloride (Extrasynthèse, Lyon, France) as external standard. The total anthocyanin amount was calculated as the sum of the individual anthocyanin concentrations. Results were also expressed as percentages of the identified peak total area. Data were subjected to analysis of variance and means were separated by the Tuckey's test. The 3-O-glucoside free anthocyanin and their acylated form percentages and the total anthocyanin amounts (table 3) were analysed by principal component analysis (PCA). Statistical analyses were performed by SAS 8.2 (SAS Institute, Cary, USA).

Results

In both years Vezza was the warmest site whereas Monforte was the freshest one; rainfall was always higher in Lessona and in Donnas whereas it was reduced in Vezza (table 1). In 2005 the ratio between rain and growing degree days (GDD) was quite similar in Donnas, Monforte and Lessona while it was lower in Vezza, due to a lower rainfall and a higher GDD. In 2004 meteorological conditions of Donnas and Lessona were similar, rainfall was lower in Monforte and in Vezza respect to Donnas and Monforte and in Vezza the accumulated GDD were the highest.
Berries reached a satisfactory level of ripening for cv. Nebbiolo; harvest was earlier in Vezza and in Monforte, later in Lessona and in Donnas. In early sites the sugar concentration was higher in both years (tables 4 and 5).
Similarly to what it has been shown for meteorological aspects the anthocyanin composition of the examined clones was more markedly different among sites in 2004 than in 2005; in the same site differences among clones were more marked in Donnas, Lessona and Monforte than in Vezza (figure 1; tables 4 and 5). The variables examined by PCA allowed to discriminate the sites and the years. On the first two principal component (Prin 1 and Prin 2) the cultivation sites were similarly separated in the two years (table 3), although the years were shifted along the abscissa axis but the sites were distributed in the same order in the two years: Donnas, Monforte, Lessona and Vezza (figure 1). In the same site the three clones were differently plotted in the two years (figure 1). Locations being equal, the two years differed in the anthocyanin concentration, which was higher in 2004 (table 4), and in the acylated anthocyanin percentages (higher in 2005; table 5).
The average total anthocyanin amount of the three clones from Donnas and Monforte was similar but in berry skins from Donnas a significantly higher peonidin-3-O-glucoside percentage and lower percentages of cyanidin-, delphinidin-, petunidin- and malvidin-3-O-glucoside were detected (tables 4 and 5). In berries from Vezza the highest malvidin-3-O-glucoside and acylated derivative percentages were detected but due to the different amounts of total anthocyanins accumulated in the different cultivation sites, this aspect did not correspond to higher concentrations (data not shown) that, on the contrary, were detected in the other sites. The total anthocyanin concentration was reduced in berry skins from Lessona and Vezza, in particular, but the percentages of peonidin-3-O-glucoside in Lessona were similar to those detected in Monforte (particularly in 2004) whereas in Vezza the peonidin-3-O-glucoside percentage was the lowest, advantaging the acylated derivatives and, sometimes, malvidin-3-O-glucoside (tables 4 and 5).
In 2004 (table 4) clones CVT 308 and CVT 142 from Vezza showed a similar anthocyanin profile but they significantly differed in the total anthocyanin amount. These two clones from Lessona, even showing a different peonidin-3-O-glucoside percentage, accumulated similar amounts of total anthocyanins, of di-substituted forms and of acetylated conjugates (data not shown). Clone CVT 308 and CVT 142 differed more markedly in Donnas and Monforte. In Donnas a similar anthocyanin amount and profile was detected for clones CVT 142 and CVT 423. In the other clone vs site combinations differences were more marked (table 4).

In 2005 (table 5) the two Picotener biotype clones (CVT 308 and 423) showed a very similar behaviour in Donnas, Monforte and Vezza whereas in Lessona clone CVT 423 presented a significantly higher peonidin-3-O-glucoside percentage to the detriment of delphinidin- and petunidin-3-O-glucoside percentages (data not shown). The Lampia biotype clone (CVT 142) was similar to the two Picotener biotype clones in Donnas whereas in Monforte and in Vezza it differed due to a lower peonidin-3-O-glucoside percentage and to a higher malvidin-3-O-glucoside percentage and, in berry skins from Vezza, also to a higher percentage of acylated forms. Clone CVT 142 also showed lower peonidin-3-O-glucoside and total acylated forms percentages in Lessona if compared to clone CVT 423.

The anthocyanin concentration was similar among clones in the same site but the same clone showed different behaviour in the different cultivation sites: clone CVT 308 anthocyanin concentration was lower in Lessona and Vezza if compared to Monforte and Donnas; clones CVT 142 and 423 anthocyanin amounts were the lowest in berries from Vezza (table 5), confirming this site as the less favourable to colour accumulation.

Conclusions

The cultivation site influenced the clone behaviour, modifying both the total anthocyanin accumulation and their percentages, thus conditioning their enological attitude. Differences in the anthocyanin amounts and profile were kept in both years and they allowed the discrimination among sites, even though the year effect was evident on the cultivation environments and on the clones. The examined clones did not present a univocal behaviour in the four sites: the two Picotener biotype clones (CVT 308 and CVT 423) showed analogies in three sites only in 2005, while the Lampia biotype clone (CVT 142) anthocyanin composition was similar to the one of the clone CVT 423 in Donnas and of the clone CVT 308 both in Donnas and in Lessona, but only in 2005.

Grapes from Vezza accumulated more sugars and less anthocyanins and they showed higher percentages of malvidin-3-O-glucoside and of total acylated derivatives respect to the other locations; this aspect would confirm the negative correlation between the anthocyanin concentration and the malvidin-3-O-glucoside percentage (R2 = – 0.65 **) that was also detected in grapes from the other observed sites. As previously pointed out by other authors (Van Leeuwen *et al*., 2004) in sandy soils (Vezza and Lessona) and in reduced rainfall conditions (Vezza), the anthocyanin concentration is low while the malvidin-3-O-glucoside percentage is the highest. The loamy soil and the cooler climate of Monforte had an opposite effect on the anthocyanin composition.

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Table 1 – Main geographical and climatic characteristics of the trial sites. Rain and GDD (growing degree days, base 10°C) refer to the period 1st April- 31st October.
(Meteorological data were supplied by Servizio Agrometereologico Regione Piemonte and by Protezione civile – Ufficio Metereologico, Regione Autonoma Valle d'Aosta).

Site	Latitude	Altitude	Rain (mm)		GDD (°C)		mm/°C	
			2004	2005	2004	2005	2004	2005
Donnas (Aosta Valley)	45°36' N	450 m	637	682	1840	1878	0.346	0.363
Monforte (Langhe area)	44°34' N	420 m	433	599	1773	1760	0.244	0.340
Vezza (Roero area)	44°45' N	350 m	312	489	1930	1925	0.162	0.250
Lessona (North-Piedmont)	45°35' N	280 m	728	636	1892	1878	0.385	0.339

Table 2 – Description of the four vineyards.

	Donnas	Monforte	Vezza	Lessona
planting	1995	1999	2000	2000
exposition	South	East	South-West	South-East
site	steep mountain area	hilly area	hilly area	flat country
soil	dry and rocky	loamy and calcareous	dry and sandy-loamy	sandy
pH	pH 6.2	pH 8.1	pH 8.2	pH 4.8
trellis system	pergola, double Guyot pruning, on terraces	vertical, Guyot pruning	vertical, Guyot pruning	vertical, Guyot pruning
vine density	2500/hectare	4600/hectare	5400/hectare	5000/hectare

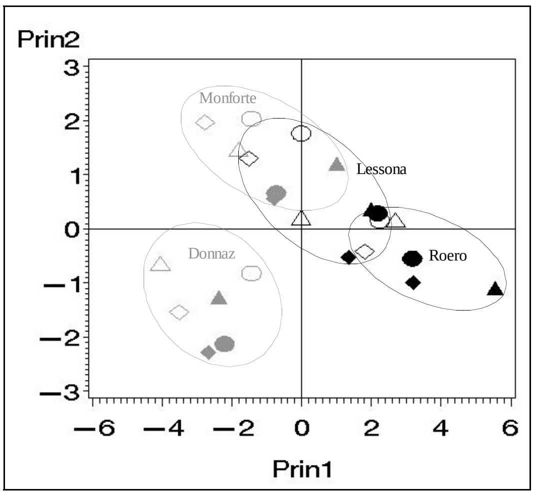

Figure 1 - Distribution of the analysed observations (clones, sites and years) on the first two principal components (Prin 1 and Prin 2).

Clones are indicated as follows: 308 = ●; 142 = ▲; 423 = ◆; empty symbols refer to 2004, filled symbols refer to 2005.

Table 3 - Eigenvectors (EV) and loadings (L) of the three principal components (Prin1, Prin2, Prin3) from PCA of Nebbiolo clones. Eigenvalues and their contribution to total variation are also listed.
Df = delphinidin-3-O-glucoside; Cy = cyanidin-3-O-glucoside; Pt = petunidin-3-O-glucoside; Pn = peonidin-3-O-glucoside; Mv = malvidin- 3-O-glucoside; tot acyl = sum of the acylated forms; tot acet = sum of the acetated derivatives; tot pcum = sum of the p-coumaroyl derivatives; tot ant = sum of the individual anthocyanins and acylated forms. Bold numbers report the variable correlated to Prin 1, 2 and 3.

	Prin1		Prin2		Prin3	
	EV	L	EV	L	EV	L
Df (%)	0.33	0.82	**0.45**	0.56	0.05	0.05
Cy (%)	-0.19	-0.46	**0.43**	0.53	**0.79**	0.71
Pt (%)	**0.35**	0.88	0.36	0.44	-0.14	-0.13
Pn (%)	**-0.37**	-0.92	-0.31	-0.39	-0.08	-0.07
Mv (%)	**0.36**	0.90	0.19	0.24	-0.39	-0.35
tot acyl (%)	**0.36**	0.89	-0.30	-0.38	0.23	0.21
tot acet (%)	0.31	0.78	-0.42	-0.52	0.27	0.25
tot pcum (%)	**0.36**	0.90	-0.25	-0.31	0.24	0.21
tot ant. (mg kg^{-1})	**-0.34**	-0.84	0.15	0.18	-0.11	-0.10
Eigenvalue	6.23		1.53		0.81	
Percent of total variance	69.3		17.0		9.0	

Table 4 - Peonidin-3-glucoside, malvidin-3-glucoside, total acylated form and total anthocyanin concentration of the three examined clones from the four cultivation sites in 2004.
SSC = soluble solid content; * = significance among clones in the same place; ** significance among sites for the same clone. Values followed by the same letter do not significantly differ at Tukey's test for $p \leq 0.05$.

2004		peonidin-3-glucoside			malvidin-3-glucoside			total acylated			total anthocyanins			SSC		
		%	*	**	%	*	**	%	*	**	mg/kg	*	**	°Brix	*	**
Donnas	308	58.0	b	a	21.0	a	a	6.1	a	b	1404	b	b	24.8	a	a
	142	67.6	a	a	9.0	b	d	4.4	c	c	1770	a	a	24.1	a	b
	423	69.4	a	a	10.3	b	d	5.3	b	b	1701	a	b	23.2	b	c
Monforte	308	48.2	b	b	21.4	a	a	4.3	b	c	1775	b	a	25.2	a	a
	142	49.7	ab	b	14.0	b	c	5.6	a	b	1269	c	b	24.8	ab	a
	423	51.7	b	b	13.0	b	c	4.7	b	c	1952	a	a	24.4	b	b
Vezza	308	40.4	b	b	25.4	ab	a	10.2	a	a	869	a	c	25.0	a	a
	142	39.0	b	c	27.4	a	a	10.3	a	a	774	b	c	25.0	a	a
	423	43.1	a	c	22.2	b	a	10.9	a	a	842	ab	d	24.9	a	a
Lessona	308	45.1	b	b	22.2	a	a	5.1	bc	c	901	b	c	24.8	a	a
	142	49.1	a	b	21.1	a	b	6.8	b	b	926	b	c	23.1	b	c
	423	50.9	a	b	15.6	b	b	5.5	b	b	1362	a	c	23.9	ab	b

Table 5 - Peonidin-3-O-glucoside, malvidin-3-O-glucoside, total acylated form percentages and total anthocyanin concentration of the three examined clones from the four cultivation sites in 2005.
SSC = soluble solid content; * = significance among clones in the same place; ** significance among sites for the same clone. Values followed by the same letter do not significantly differ at Tukey's test for $p \leq 0.05$.

2005		peonidin-3-glucoside			malvidin-3-glucoside			total acylated			total anthocyanins			SSC		
		%	*	**	%	*	**	%	*	**	mg/kg	*	**	°Brix	*	**
Donnas	308	65.0	ab	a	10.7	a	b	7.3	a	c	1178	a	a	25.0	a	a
	142	62.5	b	a	10.3	a	c	6.6	a	c	1215	a	a	22.6	c	a
	423	67.7	a	a	9.2	a	c	7.2	a	c	1319	a	a	23.4	b	b
Monforte	308	49.2	a	b	14.5	b	b	7.2	a	c	1118	a	a	24.4	a	b
	142	43.1	b	b	24.9	a	b	7.0	a	bc	1226	a	a	23.0	b	a
	423	49.5	a	b	16.1	b	b	7.1	a	c	1191	a	ab	22.8	b	c
Vezza	308	37.9	a	c	21.5	b	a	12.9	b	a	883	a	ab	24.3	a	b
	142	29.9	b	c	31.3	a	a	15.1	a	a	678	a	b	21.5	b	b
	423	38.9	a	c	22.7	b	a	12.9	b	a	825	a	c	24.3	a	a
Lessona	308	40.3	b	c	26.0	a	a	9.1	ab	b	907	a	b	21.6	b	c
	142	40.4	b	b	26.2	a	ab	8.8	b	b	947	a	ab	22.5	a	a
	423	44.9	a	a	22.5	a	a	9.5	a	b	1060	a	bc	22.5	a	c