Vitis vinifera - a chemotaxonomic approach: Anthocyanins in the skin

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Summary: The gaining of new knowledge about varietal differences in grapevines can be useful for the designing of genetic improvement programs. More and more, chemical methods complement ampelographic ones in the study of variability in grapevines.

This work is aimed at the anthocyanin profiling of red-coloured grapes, of which ca. 120 cultivars were sampled; among these there were a high number of old Italian vines and 30 *Vitis vinifera* ssp. *silvestris* originating from different areas of Italy. Anthocyanins were HPLC separated and quantified with the aid of an inverse phase microbore column and a photodiode detector.

Grapevines were numerically separated in groups using as indexes the percentage of the 5 monoglucosides present, the summations of: acetic esters; malvidin-3-monoglucoside-caffeoate plus all 5 p-coumaric esters; as well as a series of relations correlated to certain enzymatic activities necessary for the esterification of glucosides, hydroxylation and methylation in the biosynthesis of several anthocyanins. Data derived from the study of indexes of varietal enzymatic activity enable us to qualify differences between grapevines linked to the synthesis of anthocyanins. The stability of anthocyanic profiles within the same grape variety enables the use of this technique for taxonomic purposes. This research study discusses the use of this technique for classification and analysis of grape phylogenesis. An in-depth look into the relations between cultivated and wild varieties is given.

Key words: Vitis vinifera, variety of vine, Italy, berry, skin, anthocyanin, glucoside, ester, analysis, statistics, ampelography, taxonomy.

Introduction

Wulf and Nagel (1978) developed the first method of separation of pigments in Cabernet Sauvignon grapes skin by means of high pressure liquid chromatography (HPLC). Since then, anthocyanins analysis proved to be useful in grapevine classification and chemotaxonomy.

The technique has been improved further, as shown by several studies on the gaining of the first analytical data (Piergiovanni and Volonterio 1981; Di Stefano and Corino 1984; Bakker and Timberlake 1985) and also on the interpretation of anthocyanin metabolism (Roggero *et al.* 1986; Darné 1988 b).

The strong discriminating power of this technique was demonstrated by the first classifications, based on direct observation of chromatograms or parts of chromatograms: groups were assembled according to similarity of monoglucoside profiles (Wenzel *et al.* 1987).

Some of the studies were aimed at developing statistical procedures, in order to obtain more complete and systematic utilization of the data derived from analysis of grape skin anthocyanins and of the individuation of the variables-set more suitable to these purposes (Scienza *et al.* 1985; Roggero *et al.* 1988).

From these works the unanimous opinion that the anthocyanin profile of grape skins can complement ampelographic methods in the study of grapevine variability was derived. This knowledge can be very useful for the development of genetic improvement programs.

Lately, an analogous application was suggested for anthocyanins of leaves of *Vitis* genus and *Vitis vinifera* varieties (DARNÉ 1988 a; DARNÉ et GLORIES 1988).

In Italy, at S. Michele Institute, a databank containing analyses of skin anthocyanins of many grape varieties was constituted and these data were used for chemotaxonomic and phylogenetic studies on some red-coloured grapes typical of Trentino Alto-Adige (SCIENZA *et al.* 1989).

This report is aimed at the presentation of the analytic and methodologic work on which the classification technique is based.

For a further examination of the taxonomic and phylogenetic implications of this report, and of its connections with other chemotaxonomic techniques, please refer to other parts of this research work (SCIENZA *et al.* 1989).

Materials and methods

In order to obtain a classification of anthocyanins we sampled technologically ripe grapes from approx. 120 varieties (Table 1). The samples were chosen among the most significant varieties from the taxonomic point of view, and they included a high percentage of old Italian vines. Approx.

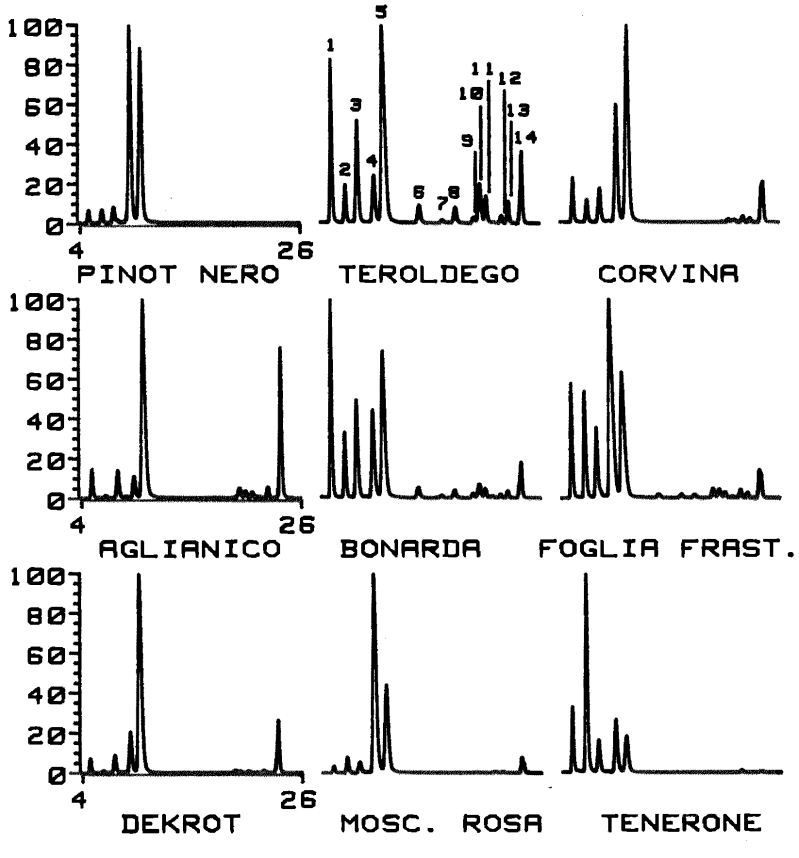

Fig. 1: Chromatographic patterns of grape skin anthocyanins of some cultivars. Time as min; 1 = delphinidin-3-glucoside, 2 = cyanidin-3-glucoside, 3 = petunidin-3-glucoside, 4 = peonidin-3-glucoside, 5 = malvidin-3-glucoside, 6-10 = (1-5)-acetates, 11-14 = (1-5)-p-coumarates.

30 *V. vinifera* ssp. *silvestris* grapevines originating from various Italien regions were also sampled. We studied a total number of 500 samples harvested in the years 1986, 1987 and 1988 primarily from North Italian ampelographic collections. Some samples were taken directly from country vineyards.

These samples were subjected to spectrophotometric determination of total skin anthocyanins, and anthocyanins were HPLC separated and quantified.

The skins of 20 frozen berries were extracted in two phases for 12 h with 100 and 50 ml of methyl alcohol, HCl 0.1 %. The extract was evaporated to dryness in a rotary evaporator at 36 °C and redissolved with a solution suitable for injection in HPLC.

The determination of total anthocyanins was made spectrophotometrically at 520 nm, with the method based on differences in pH.

The separation of single anthocyanins was made by means of gradient elutions using a chromatograph Hewlett Packard 1090M with diode-array detector HP 1040 and column type C18 Hypersil ODS (5 μm, 200 x 2.1 mm). Eluants were: A = perchloric acid 0.3 %, B = methanol. Identification was made according to retention times and UV-VIS spectra of each peak. Quantification was made on areas at 520 nm (Fig. 1).

Data thus obtained were statistically processed using the statistic package SPSS-X.

Results and discussion

A classification of cultivated varieties was thus obtained; as a second step we also evaluated the resemblance of wild varieties to cultivated ones.

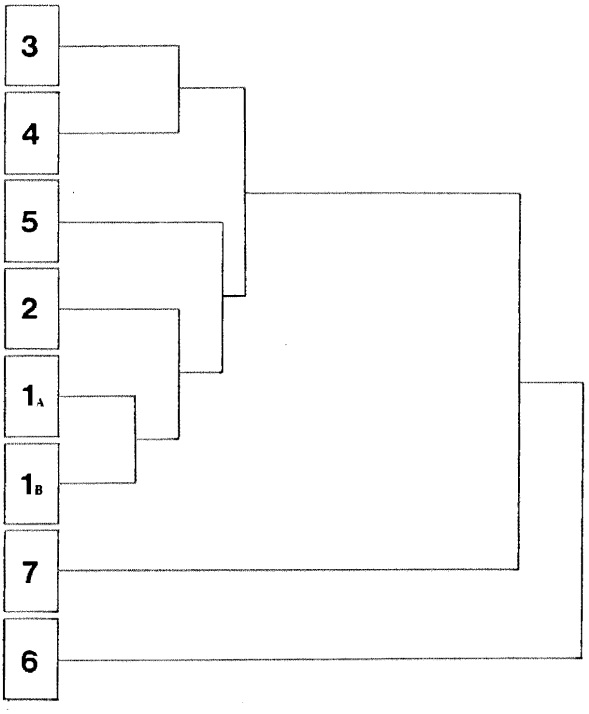

Fig. 2: Classification of cultivated varieties obtained by cluster analysis.

Table 1: List of varieties subjected to the present classification. They are divided into groups according to results of cluster analysis

GROUP 0: Pinot nero, Pinot grigio, Pinot*Dekrot, Pinot tete de negre.
GROUP 1-a: Ancellotta, Barbera, Bombino, Braubana, Cabernet franc, Cabernet sauvignon, Cabrusina, Codelonghe, Colorino pisano, Croatina, Fortana, Fumat, Fumin, Givan, Lagrein, Lambrusco di Sorbara, Lambrusco grasparossa, Lambrusco maestri, Lambrusco salamino, Malbo gentile, Malvasia di Casorzo, Malvasia nera di Lecce, Malvasia nera di Pisa, Mariabino, Marzemino, Merlot, Negrat, Nera grossa, Petit Verdot, Refoscone, Ribolla nera, Teroldego, Vien de nus, 107-2 (Merlot x Marzemino), 107-3 (Merlot x Marzemino), 95-5(Cab.Franc x Merlot).
GROUP 1-b: Aleatico, Bonamico, Burghisana, Canaiolo, Cesanese comune, Ciliegiolo, Colorino, Corvina, Fortana nera (Brugnola), Gamay, Grillone, Kolor, Lambrusco di Alessandria, Lambrusco marani, Moscato violetto, Mourvedre, Negrara, Neyret, Pomela schiava, Rafosal, Rondinella, Rossara, Uvarosa, 200-496.
GROUP 2: Aglianico, Albanina, Aramon, Balsamina, Canena, Cornacchia, Groppello ruberti, Malbech, Negretto, Pavana, Schiava lombarda, Syrah, Tosca, Turca, Incrocio Bruni 147.
GROUP 3: Bonarda, Brugnola, Casetta, Corvino, Cuneute, Denela, Dindarella, Forgiarin, Jagodinka, Lambrusco oliva, Molinara, Oseleta, Pelara, Picolit nero, Pignul, Quaiara, Rossetta di montagna, Rossiola, Simesara, Sangiovese (Brunello), Sangiovese (Prugnolo), Sangiovese (Chianti g.n.), Sangiovese (Chianti p.), Uva d'oro, Vercluna.
GROUP 4: Cianorie, Colorino di Lucca, Foglia frastagliata, Forselina, Groppello, Malvasia nera di Brindisi, Rossignola.
GROUP 5: Dekrot, Tocai rosa.
GROUP 6: Mammolo pisano, Moscato d'Adda, Moscato rosa, Muscat rouge, Nebbiolo, Schiava gentile, Schiava grossa, Trollingher.
GROUP 7: Tenerone.

Among the varieties studied, the Pinot group was singled out as the only one different from the qualitative point of view, as this cultivar lacks esterificated anthocyanins. For this reason, this variety was not included in further elaborations.

Table 2: The 6 canonical discriminant functions

FUNCTION	EIGENVALUE	PERCENT OF VARIANCE	CUMULATIVE PERCENT	CANONICAL CORRELATION
1*	12.05263	72.57	72.57	0.9609303
2*	2.84243	17.11	89.68	0.8600860
3*	1.31966	7.95	97.63	0.7542566
4*	0.34797	2.10	99.72	0.5080804
5*	0.04530	0.27	99.99	0.2081786
6*	0.00114	0.01	100.00	0.0337203

STRUCTURE MATRIX:
POOLED WITHIN-GROUPS CORRELATIONS BETWEEN DISCRIMINATING VARIABLES AND CANONICAL DISCRIMINANT FUNCTIONS
(VARIABLES ORDERED BY SIZE OF CORRELATION WITHIN FUNCTION)

	FUNC 1	FUNC 2	FUNC 3	FUNC 4	FUNC 5	FUNC 6
V4	0.63193*	0.40868	-0.16058	0.44765	-0.23703	-0.34903
V2	0.28959	-0.82135*	-0.47218	0.03277	-0.02424	-0.08845
SCINNAM	-0.41314	0.49538	-0.65091*	-0.25071	-0.04214	0.30588
V3	-0.02890	-0.41004	0.35193	-0.66091*	0.03317	0.08413
V1	0.00087	-0.45684	0.32305	-0.64191*	-0.10481	0.51300
V5	-0.36503	0.17682	0.39852	0.63652*	-0.18336	-0.46652
SACILATI	-0.10245	0.04917	0.11299	-0.23219	0.81604*	0.41544

UNSTANDARDIZED CANONICAL DISCRIMINANT FUNCTION COEFFICIENTS

	FUNC 1	FUNC 2	FUNC 3	FUNC 4	FUNC 5	FUNC 6
V1	0.5944021	0.1188802	0.1547636	1.600972	-0.1666806	2.478363
V2	0.5883325	-0.3051396E-01	-0.1163867	1.675948	0.7740529E-01	2.257265
V3	0.6668733	0.2861497	0.6093347E-01	1.258827	0.1842856	1.942293
V4	0.7505337	0.2667792	0.5683841E-01	1.569709	0.1818205E-01	2.267260
V5	0.5054739	0.1614975	0.9598790E-01	1.664238	0.1458670E-01	2.266669
SACILATI	0.6041821	0.1926011	0.7046074E-01	1.571663	0.2705803	2.242297
SCINNAM	0.5232926	0.2710039	-0.8984168E-01	1.583276	-0.1125723E-01	2.379620
(CONSTANT)	-58.26904	-19.06001	-5.104143	-158.0238	-2.546739	-226.0329

Classification of cultivated varieties

Classification was made according to the seven following variables: the five monoglucoside concentrations (expressed as percentage chromatographic area at 520 nm), the summation of acetic esters and the summation of p-coumaric esters. The summations of the two kinds of esters were utilized, according to the hypothesis – verified as far as acetic esters are concerned (Wenzel *et al.* 1987) – that the esters syntheses rate from the anthocyanin-3-monoglucosides varies only slightly.

We obtained a mean anthocyanic profile for every grapevine variety from which we had available analyses over different years, origins and clones.

These data were processed in order to make a research on typologies. Classification was obtained by means of cluster analysis, following the method 'average linkage between groups'. As to the proximity measures, we used the squares of Euclidean distances.

The cultivars were thus divided into 7 groups. Their classification obtained is shown in Table 1 and in the corresponding dendrogram (Fig. 2).

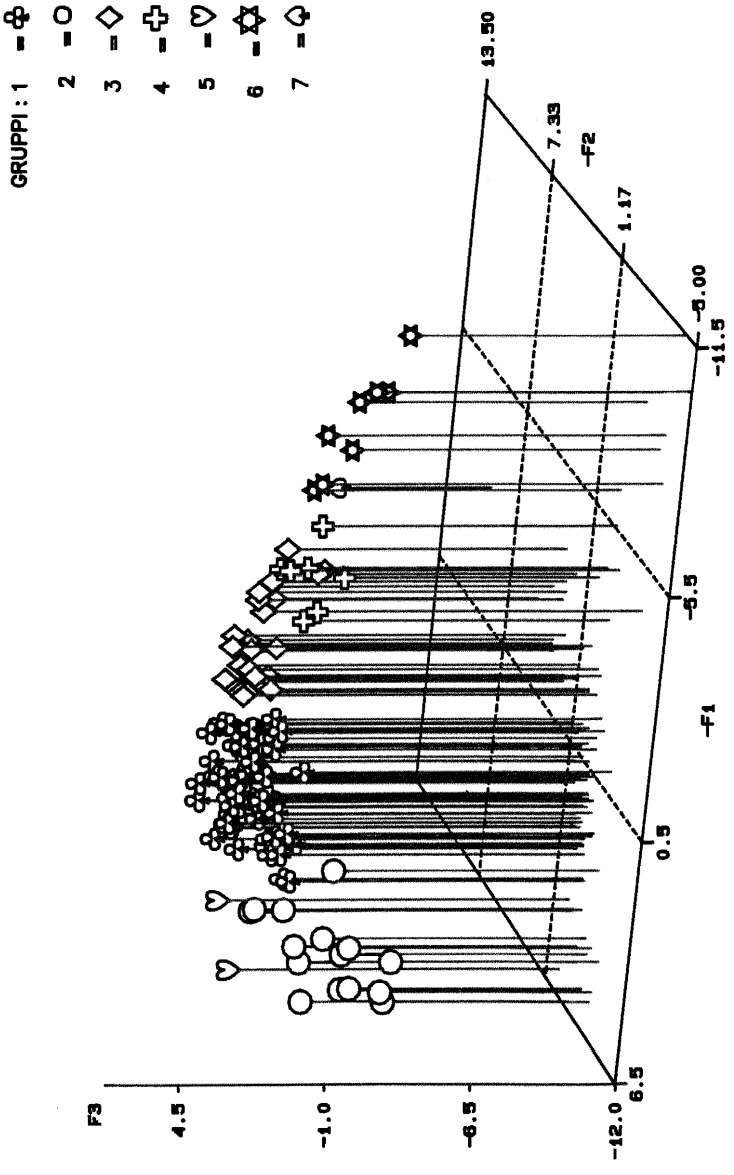

Fig. 3. Subdivision of cultivated varieties into 7 groups by means of discriminant analysis. Each symbol represents one cultivar.

Table 3: Classification results for cases not selected for use in the analysis

ACTUAL GROUP		NO. OF CASES	PREDICTED GROUP MEMBERSHIP 1	2	3	4	5	6	7
GROUP	1	170	159	4	1	3	3	0	0
			93.5%	2.4%	0.6%	1.8%	1.8%	0.0%	0.0%
GROUP	2	17	1	15	0	0	1	0	0
			5.9%	88.2%	0.0%	0.0%	5.9%	0.0%	0.0%
GROUP	3	219	21	0	190	0	2	0	6
			9.6%	0.0%	86.8%	0.0%	0.9%	0.0%	2.7%
GROUP	4	13	1	0	1	11	0	0	0
			7.7%	0.0%	7.7%	84.6%	0.0%	0.0%	0.0%
GROUP	5	2	0	0	0	0	2	0	0
			0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%
GROUP	6	10	0	0	0	1	0	9	0
			0.0%	0.0%	0.0%	10.0%	0.0%	90.0%	0.0%
GROUP	7	1	0	0	0	0	0	0	1
			0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	100.0%

PERCENT OF "GROUPED" CASES CORRECTLY CLASSIFIED: 89.58%

The subdivision into groups obtained in this way was further confirmed by discriminant analysis. The discriminant analysis was carried out following the 'stepwise' method based on Wilks' lambda; we used the seven parameters formerly used for the cluster analysis. With this method we obtained six linear canonic discriminant functions. Some of the most important characteristics of this elaboration are reported in Table 2:

The first three functions can account for 97.6 % of total variance. The first discriminant function (F1) accounts for 72.6 % of variance and is well correlated (0.632) to peonidin-3-monoglucoside.

The second discriminant function (F2) explains 17.1 % of variance, and is inversely correlated (-0.821) to cyanidin-3-monoglucoside.

The third function (F3) explains 7.9 % of variance and is inversely correlated (-0.651) to the summation of p-coumaric esters.

The six canonic discriminant functions thus obtained confirm 95.8 % (i. e. in 113 cases out of 118) of the subdivision obtained using cluster analysis.

The distribution of the cultivars in the space defined by the first three canonic discriminant functions is shown in Fig. 3.

The classification of the 118 cultivars into 7 groups explained above was obtained using mean anthocyanin profiles. We decided to evaluate reliability of these results by assigning the 432 samples stored in our databank to these seven groups. The division into seven typologies was confirmed in 89.6 % of the cases (i. e. in 387 cases out of 432), as shown in Table 3.

Table 4: Mean composition parameters of Sangiovese grapes sampled in the years 1987 and 1988 in different areas of Tuscany. The single anthocyanins are expressed as percentage areas at 520 nm: the total anthocyanins appear as malvidin diglucoside chloride (mg/100 g of grapes)

	BRUNELLO (N=53)		PRUGNOLO (N=46)	
Parameter	Mean Conc.	Standard Deviation	Mean Conc.	Standard Deviation
Dp	11.22	3.13	13.54	3.42
Cy	21.82	6.28	18.92	5.31
Pt	12.99	2.42	15.15	2.66
Pn	18.63	5.21	14.66	4.94
Mv	33.74	7.83	36.19	6.03
Sum acet.	00.28	0.10	00.26	0.10
Sum coum.	01.24	0.45	01.21	00.27
Total conc.	110.4	66.9	132.0	81.2
	CHIANTI P. (N=26)		CHIANTI G.N. (N=67)	
Parameter	Mean Conc.	Standard Deviation	Mean Conc.	Standard Deviation
Dp	12.40	2.22	11.83	3.29
Cy	18.88	4.18	18.10	7.05
Pt	14.60	1.51	14.08	2.14
Pn	15.85	2.73	15.79	3.61
Mv	36.59	6.54	38.58	11.01
Sum acet.	00.22	00.07	00.21	00.08
Sum coum.	01.41	00.62	01.35	00.64
Total conc.	50.9	12.6	126.0	89.1

This outcome proved that classification obtained through mean anthocyanin profiles is sufficiently valid even for identification of single samples.

A distinguishing characteristic of each cultivar is the variability of anthocyanin profiles between individual samples. In order to illustrate this difference in behaviour, we show in Fig. 4 the classification of 63 samples belonging to the Teroldego variety (this grapevine is cultivated in a circumscribed and homogeneous area) and 194 samples of Sangiovese (cultivated in an area much wider both from the geographical and climatic point of view). This figure clearly shows that the variability range of Sangiovese is much wider than that of Teroldego.

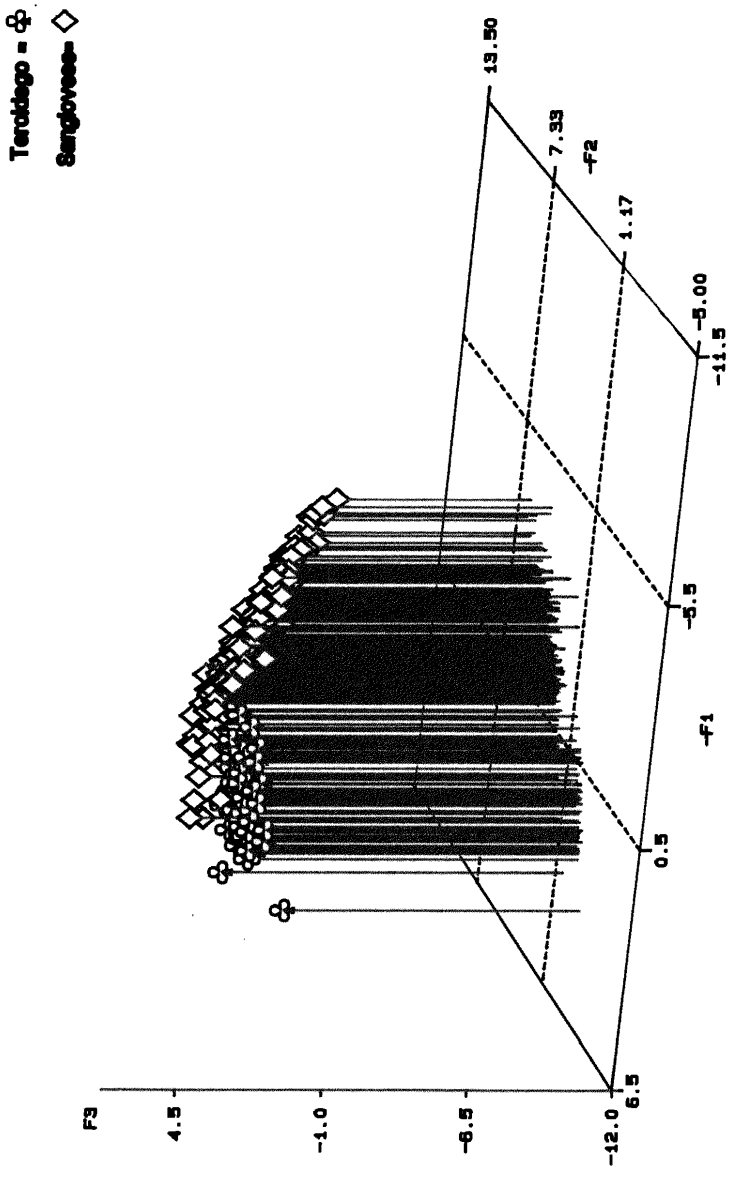

Fig. 4: Variability range of anthocyanin profiles of cvs Sangiovese and Teroldego. F1, F2 and F3 are the first three discriminant functions utilized for classification of cultivated varieties.

Table 4 shows how a sufficiently high number of samples can lead to average anthocyanin profiles extremely similar even when working on different clones of the same cultivar. This table refers to Sangiovese grapes sampled in the years 1987 and 1988 in different areas of Tuscany. This grape underwent various selections over the years, which resulted in a remarkable polymorphism and to the consequent attribution of different names (Brunello, Montepulciano, Prugnolo, Sangiovese, Sangioveto).

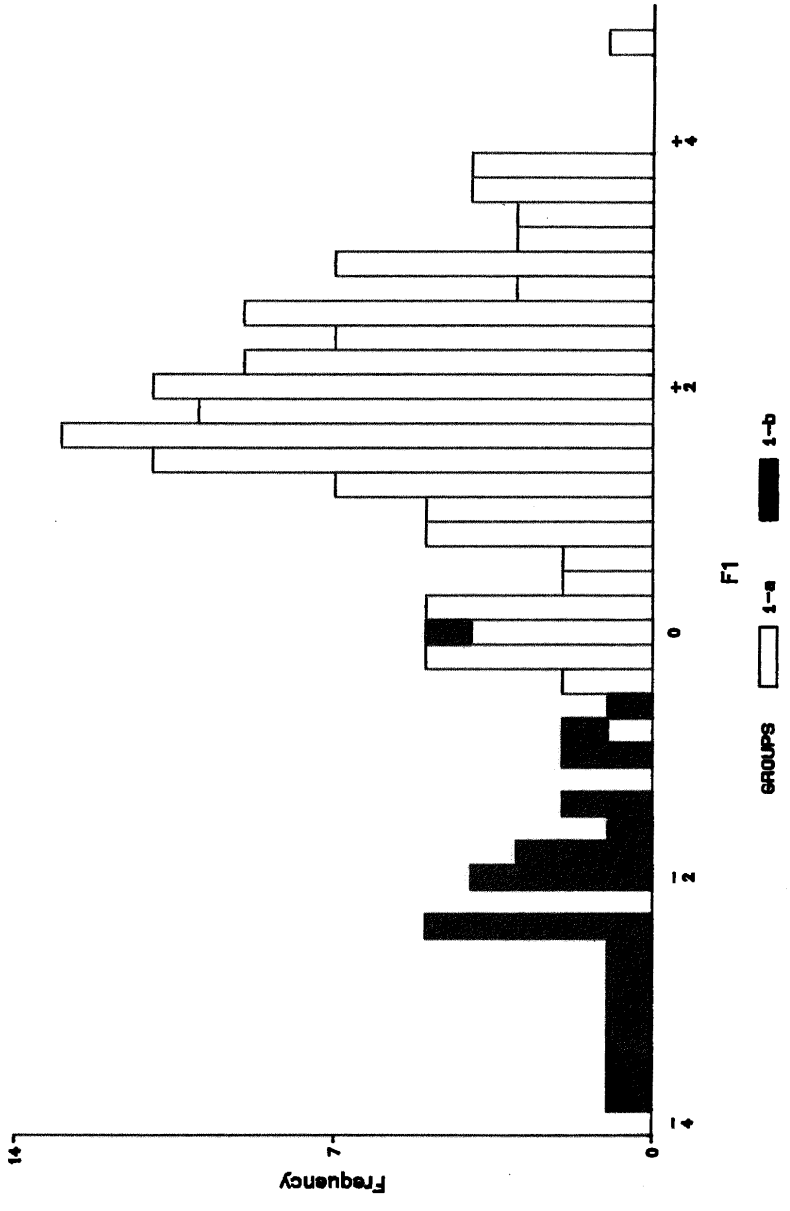

Fig. 5: Separation of groups 1-a and 1-b by means of linear discriminant analysis.

The similarity of these samples is such that they have been assigned to the same group (No. 3), as shown in Table 1, and in the cluster analysis of mean profiles these cultivars are placed as nearest neighbours.

Further subdivision can be obtained by studying the groups singled out one by one.

For example, a cluster analysis was performed on the cultivars belonging to group No. 1, the largest among the 8 groups identified so far (7 plus Pinot).

We were able to further divide this group into two sub-groups, shown in Table 1 with the codes 1-a and 1-b. Discriminant analysis of these two sub-groups resulted in a correct classification in 97.7 % of the samples (i. e. 166 out of 170), as shown in Fig. 5.

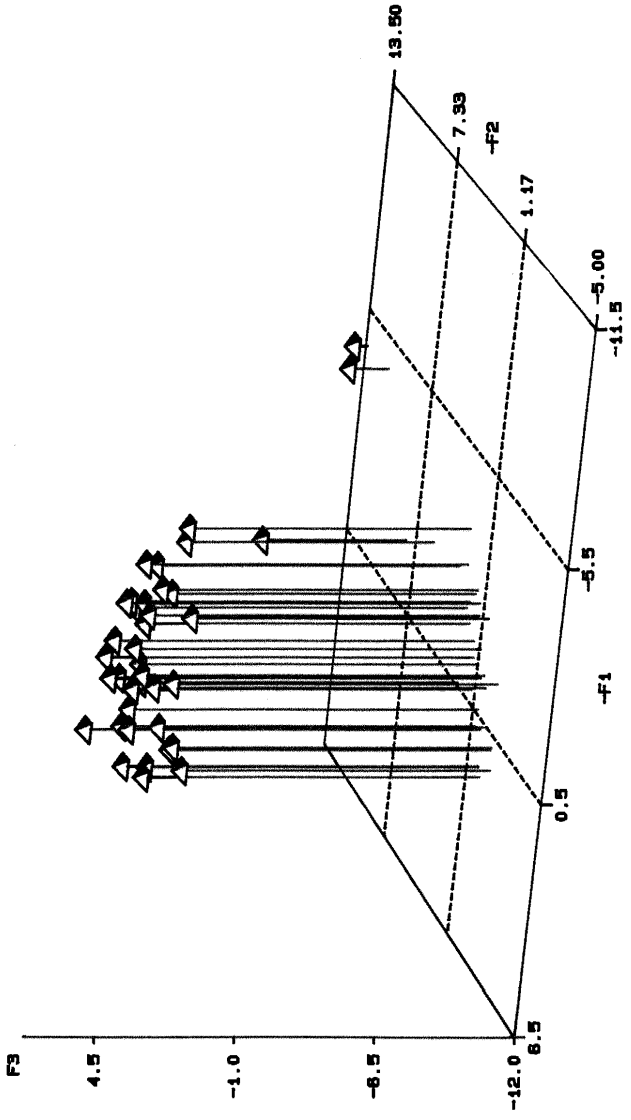

Fig. 6: Distribution of *Vitis vinifera* ssp. *silvestris* samples originating from various Italian regions in the space defined by the first three discriminant functions. The functions are the same as utilized for classification of cultivated varieties.

Vitis vinifera ssp. *silvestris*

The analysis of anthocyanins in wild grape samples originating from different areas of Italy revealed a wide range of anthocyanic profiles. At present, not the whole range shown in cultivated varieties is covered by wild varieties, but this is probably due to the fact that the number of wild samples examined is considerably lower than that of cultivated ones.

47 samples coming from 30 *V. vinifera* ssp. *silvestris* were plotted within the space defined from the three canonic discriminant functions previously calculated. Their distribution covers a considerable space, as shown in Fig. 6.

Conclusions

This research on anthocyanin profiles of approx. 500 samples belonging to about 120 cultivated varieties and 30 *V. vinifera* ssp. *silvestris* resulted in a subdivision of samples into 9 groups.

The utilization of percentages (instead of absolute quantities) reduces the influence of variability due to phenotype on classification (synthesis of different absolute quantities connected to ripening phase and year).

The utilization of percentages also allows a better verification of similarities between varieties belonging to the same family, often very different from one another as far as the absolute quantities of anthocyanins are concerned, but with similar profiles (see Moscati).

The seven variables suggested are homogeneous and consequently a standardization is not necessary. This procedure allows avoidance of possible loss of information consequent to standardization.

In Table 5 the mean composition of the parameters of the groups studied is shown. In this table, besides the percentage composition and the total anthocyanins, a series of relations supposed to be correlated to certain enzymatic activities necessary for the esterification of glucosides (Ratio 2 and Ratio 5), hydroxylation (Ratio 1) and methylation (Ratio 3 and Ratio 4) in the biosynthesis of several anthocyanins are shown.

These relations, within each one of the 9 typologies, show a dispersion of values higher than that of the initial concentrations from which they derived, depending on the way groups were constituted.

It can be clearly seen that the two 'methylation indexes' of tri- and di-substituted, although they have different absolute values, are generally covariant.

The formation of acetic esters and p-coumaric esters seems to be independent from one another, as can be inferred from the remarkable variability of the ratio between the two esters (Ratio 2).

The examination of the values of the variables peonidin-3-monoglucoside, cyanidin-3-monoglucoside and summation of p-coumaric esters shows the strong correlation between these factors and our first three discriminant functions, and consequently their importance as differentiating factors.

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Table 5: Mean composition parameters of the groups. The single anthocyanins are expressed as percentage areas at 520 nm; the total anthocyanins appear as malvidin-diglucoside chloride (mg/100 g of grapes)

	GROUP 0 (N=4)			GOUP 1-a (N=36)		
Parameter	Mean Conc.	Std. Dev.	Notes	Mean Conc.	Std. Dev.	Notes
Dp	03.16	1.08		14.34	3.75	(++)
Cy	02.09	0.83	(-)	03.66	1.93	
Pt	05.12	1.37		11.53	2.91	(++)
Pn	35.07	10.31	(+)	08.57	3.95	(-)
Mv	54.55	11.00	(++)	38.45	4.05	
Sum acet.	00.00		(---)	08.90	5.04	(+++)
Sum coum.	00.00		(---)	14.17	4.12	(+)
Total conc.	106.9	94.1		214.7	101.0	
Ratio 1						
(tri)/(di)	01.94	1.13		06.51	3.37	(+)
Ratio 2						
(Acet/Coum.)	***	***		00.68	0.47	(+++)
Ratio 3						
(Mv/Dp)	19.16	8.28	(++)	02.94	1.08	(-)
Ratio 4						
(Pn/Cy)	17.75	3.73	(++)	2.68	1.20	(-)
Ratio 5						
(Esters/Free)	00.00	0.00	(---)	00.31	0.13	(++)

	GROUP 1-b (N=24)			GROUP 2 (N=15)		
Parameter	Mean Conc.	Std. Dev.	Notes	Mean Conc.	Std. Dev.	Notes
Dp	05.56	2.28		04.55	2.02	
Cy	02.95	1.53		00.63	0.22	(--)
Pt	06.77	2.73		05.78	2.14	
Pn	19.09	6.51		04.60	1.66	(---)
Mv	48.49	4.93	(+)	43.89	6.98	
Sum acet.	02.75	2.37		06.49	3.36	(++)
Sum coumar.	13.65	5.13		32.62	7.50	(+++)
Total conc.	096.7	68.4		106.6	77.4	
Ratio 1						
(tri)/(di)	03.09	1.19		11.36	3.85	(++)
Ratio 2						
(Acet/Coum.)	00.20	0.16		00.21	0.12	(--)
Ratio 3						
(Mv/Dp)	10.64	5.43		11.74	6.12	
Ratio 4						
(Pn/Cy)	08.28	5.53		07.84	2.83	
Ratio 5						
(Esters/Free)	00.20	0.09		00.69	0.22	(+++)

(Continued overleaf)

Table 5 (continued)

	GROUP 3 (N=25)			GROUP 4 (N=7)		
Parameter	Mean Conc.	Std. Dev.	Notes	Mean Conc.	Std. Dev.	Notes
Dp	16.14	5.07	(+++)	07.41	3.64	(-)
Cy	14.33	6.39	(++)	09.73	4.37	(+)
Pt	12.32	2.32	(+++)	06.57	2.43	(-)
Pn	18.77	5.13		38.19	5.63	(++)
Mv	28.28	6.99		27.06	4.76	(-)
Sum acet.	03.64	3.56	(+)	02.22	1.46	
Sum coumar.	06.26	2.97		08.47	2.88	
Total conc.	167.6	123.3		124.4	90.3	
Ratio 1 (tri)/(di)	01.84	0.58		00.87	0.18	(-)
Ratio 2 (Acet/Coum.)	00.55	0.48	(++)	00.28	0.18	(+)
Ratio 3 (Mv/Dp)	02.00	0.93	(--)	05.90	6.77	
Ratio 4 (Pn/Cy)	01.77	1.32	(--)	06.75	8.57	
Ratio 5 (Esters/Free)	00.11	0.07		00.12	0.04	

	GROUP 5 (N=2)			GROUP 6 (N=8)		
Parameter	Mean Conc.	Std. Dev.	Notes	Mean Conc.	Std. Dev.	Notes
Dp	02.27	0.44	(--)	01.55	0.98	(---)
Cy	00.39	0.20	(---)	07.99	3.86	
Pt	03.91	0.56	(--)	03.03	1.30	(---)
Pn	07.48	4.69	(--)	62.54	6.78	(+++)
Mv	68.20	1.19	(+++)	18.00	8.85	(--)
Sum acet.	01.36	0.65	(-)	01.42	1.05	
Sum coumar.	15.68	3.77	(++)	05.23	2.34	(-)
Total conc.	105.8	92.5		052.2	34.2	
Ratio 1 (tri)/(di)	11.73	7.31	(+++)	00.33	0.16	(---)
Ratio 2 (Acet/Coum.)	00.08	0.02	(---)	00.24	0.16	
Ratio 3 (Mv/Dp)	30.67	6.45	(+++)	13.72	6.58	(+)
Ratio 4 (Pn/Cy)	18.50	2.63	(+++)	10.39	6.39	(+)
Ratio 5 (Esters/Free)	00.21	0.07	(+)	00.07	0.04	(-)

(Continued overleaf)

Table 5 (continued)

	GROUP 7 (N=1)		
Parameter	Mean Conc.	Std. Dev.	Notes
Dp	13.25		(+)
Cy	47.50		(+++)
Pt	09.09		(+)
Pn	15.65		
Mv	13.10		(---)
Sum acet.	00.24		(--)
Sum coumar.	01.10		(--)
Total conc.	020.8		
Ratio 1 (tri)/(di)	00.56		(--)
Ratio 2 (Acet/Coum.)	00.22		(-)
Ratio 3 (Mv/Dp)	00.99		(---)
Ratio 4 (Pn/Cy)	00.33		(---)
Ratio 5 (Esters/Free)	00.01		(--)

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