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Biochemical features of native red wines and genetic diversity of the corresponding grape varieties from Campania region

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ABSTRACT

Campania region has always been considered one of the most appreciated Italian districts for wine production. Wine distinctiveness arises from their native grapevines. To better define the chemical profile of Campania autochthonous red grape varieties, we analysed the phenolic composition of Aglianico di Taurasi, Aglianico del Vulture, Aglianico del Taburno, Piedirosso wines, and a minor native variety, Lingua di Femmina in comparison with Merlot and Cabernet Sauvignon, as reference cultivars. A genetic profiling was also carried out using microsatellite molecular markers with high polymorphic and unambiguous profiles. Principal component analysis applied to 72 wines based on the 18 biochemical parameters, explained 77.6% of the total variance and highlighted important biological entities providing insightful patterns. Moreover, comparison of SSR-based data with phenylpropanoid molecules exhibited a statistically significant correlation. Our approach might be reasonably adopted for future characterisations and traceability of grapevines and corresponding wines.

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1. Introduction

Grapes (*Vitis vinifera* L.) belong to the world's largest fruit crops, as they cover a total area of 7.6 million hectares, with a global production of around 68 million tonnes in 2010 (International Organisation of Vine, 2012). The greatest proportion of grapes is employed for wine production, likely the most important use of grapes in terms of tradition, literature and even religion. According to OIV Statistical Report on World Vitiviniculture (International Organisation of Vine & Wine, 2012), in 2010 about 265 million of hectoliters of wine were produced worldwide, 20 million of which only in Europe, with Italy (48.5 Mhl), France (45.7 Mhl), and Spain (35.2 Mhl) as the world's leading wine-producing countries. In Italy, grapevines cover an area of about 800,000 ha, unevenly distributed all over the country. Campania region, due to its particular climatic conditions and fertility of its soil, is historically considered one of the most appreciated Italian districts for wine production. Campania wine distinctivenesses arise from the many autochthonous red grape varieties, such as Aglianico biotypes ('Aglianico di Taurasi', A. del Vulture' and 'A. del Taburno') and 'Piedirosso'. In addition, a series of minor, still not-well characterised, grape varieties is gaining an increasing interest. Given the renewed quality of their wines, it appears of particular relevance for the wine industry and marketing to preserve the valuable traits linked to genetic constitution, geographical origin of production and unique vinification technologies. In addition, the European Union is particularly interested in agricultural productions that combine safety and quality attributes with a clear regional identity (EC regulations 2081/92 and 1898/06). Attention to food authentication is due to several reasons, including health, media attention, specific organoleptic qualities of regional products (Luykx, Peters, van Ruth, & Bouwmeester, 2008).

In this scenario, the efficient assessment of food products authenticity is a major challenge for both producers and consumers. Organic and inorganic wine constituents (Kment et al., 2005), as well as wine sensory attributes, are usually used to distinguish wines according to vinification technology, area of origin and variety. Polyphenols are a class of grape organic compounds that confer wine important sensory properties as appearance, taste and mouthfeel. In particular, anthocyanins are mainly responsible for colour and proanthocyanins for bitterness and astringency (Arnold, Noble, & Singleton, 1980). In addition, many polyphenolic compounds display significant benefits to human nutrition and health (Bozan, Tosun, & Özcan, 2008). According to Ribéreau-Gayon (1982), the phenolic fingerprint is typical of each individual cultivar so that the analysis of anthocyanins and flavonoids has been used to distinguish grape varieties (Berente, García,

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Reichenbächer, & Danzer, 2000). Anthocyanin composition, in particular, is quite distinctive and its determination can be a parameter to assess grape authenticity (Revilla, Garcia-Beneytez, Cabello, Martin-Ortega, & Ryan, 2001). Due to the importance of polyphenols in determining the overall grape and grape-derived products qualities as well as in differentiating grape cultivars, considerable effort has been made in determining the compositions and contents of polyphenolic compounds in grapes, as well in wine (Guerrero et al., 2009a).

Along with the chemical profiling, molecular biology techniques offer powerful analytical tools for grape variety identification and product authentication. In particular, molecular markers are irrespective of environmental factors, free of epistatic interactions and pleiotropic effects, objectively analyzable at all stages of plant growth, and therefore they provide an excellent tool for fingerprinting and assessing genetic variation and relatedness among cultivars of various crops (Kalia, Rai, Kalia, Singh, & Dhawan, 2011). In recent years, several molecular markers has become available for basic and applied studies; random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs) are some of the markers developed for germplasm characterisation of different crop plants (Varshney et al., 2007). In particular, SSRs, also known as microsatellites, are useful for many applications in plant genetics and breeding because of their reproducibility, multiallelic nature, codominant inheritance, relative abundance and good genome coverage. They have extensively been used in grape for analysing the genetic diversity of local accessions and exploring pedigree authentication, often in combination with biochemical analyses. Albeit the interesting results reported in literature, no attempts to directly correlate genetic profiles and wine analytical composition have been carried out.

In this work we assessed the phenolic content and composition of wines obtained from native Campania region red grape varieties as Aglianico biotypes (Taurasi, Vulture and Taburno), Piedirosso and Lingua di Femmina. We also carried out the genetic analysis of these cultivars to acquire detailed information for a certification of their production authenticity that combines quality attributes with a clear regional identity.

2. Materials and methods

2.1. Wines and plant material sampling

Wines were obtained from grapes of *V. vinifera* cultivar 'Piedirosso' (P), 'Cabernet Sauvignon' (CS), 'Merlot' (M), 'Aglianico di Taurasi' (T), 'Aglianico del Taburno' (A), 'Aglianico del Vulture' (V) and 'Lingua di Femmina' (L). All grapes and leaf samples were collected from vineyards located in the area surrounding the city of Benevento (Campania, Italy). These vineyards lay at an altitude ranging between 350 and 650 m above sea level (a.s.l.); the annual mean precipitation received was 850 mm, and the annual mean temperature 13 °C. The soils were both clays and limestone. The vineyards were located in the Taburno DOC area of Campania region (latitude between 41° and 48°N, longitude between 12° and 14°W). Leafs were collected from individual plant. All wines were produced by a conventional winemaking procedure. Briefly grapes were destemmed and crushed, the must was treated with $K_2S_2O_5$ (60 mg/kg of grapes). Fermentation took place at almost 26 °C with indigenous yeast and the cap was immersed twice a day. Maceration of the pomace lasted 18–20 days then the must was pressed and the finished wines obtained. Wines were stored in stainless steel tanks and analysed 6–10 months after the end of the fermentation. Overall, 72 wines were analysed for 18 biochemical parameters.

2.2. Biochemical analysis

2.2.1. Standard chemical analyses and spectrophotometric measurements

Tannins were evaluated as described by Ribéreau-Gayon and Stonestreet (1966). Total anthocyanins and Vanillin Reactive Flavans (VRF) were determined according to Di Stefano and Guidoni (1989). Colour intensity and hue were evaluated according to Glories (1984) methods. A Shimadzu UV-1800 (Kyoto, Japan) UV spectrophotometer was used for all data pertaining to the results reported in this article. Photometric accuracy was of ±0.002 Abs and photometric repeatability was less than ±0.001 Abs. All analyses were carried out in triplicate.

2.2.2. HPLC equipment and chemicals

A HPLC Shimadzu LC10 ADVP apparatus was used (Shimadzu Italy, Milan), consisting of a SCL-10AVP system controller, two LC-10ADVP pumps, a SPD-M 10 AVP detector, and an injection system full Rheodyne model 7725 (Rheodyne, Cotati, CA) equipped with a 20 μL loop. All the samples were filtered through 0.45 μm, Durapore membrane filters (Millipore – Ireland) into glass vials and immediately injected into the HPLC system. All chromatographic solvents were HPLC ultra gradient grade and were purchased from Merck (Darmstadt, Germany). Malvidin-3-monoglucoside (Mv-3-glc), *trans*-resveratrol, quercetin (Sigma–Aldrich, Milan, Italy), (+)-catechin hydrate (purity >90%) and (−)-epicatechin (purity ⩾90%) (Fluka, Milan, Italy) standards were used in this study.

2.2.3. Anthocyanins method

For the separation and quantification of anthocyanins a Waters Spherisorb column (250 × 4.6 mm, 4 μm particles diameter) with precolumn (NOVA-PAK C18 20 × 3.9 mm, 4 μm particles diameter) was used. HPLC separation of anthocyanins was carried out according to the OIV Compendium of International Methods of Wine and Must Analysis (2007) with slight modifications. Twenty μl of wine or calibration standards were injected onto the column. The HPLC solvents were: solvent (A) water/formic acid/acetonitrile (87:10:3) v/v; solvent (B) water/formic acid/acetonitrile (40:10:50) v/v. Zero-time conditions were 94% A and 6% B, after 15 min the pumps were adjusted to 70% A and 30% B, at 30 min to 50% A and 50% B, at 35 min to 40% A and 60% B, at 41 min, end of analysis, to 94% A and 6% B. This zero-time solvent mixture was followed by 10-min equilibrium period prior to inject the next sample. The flow rate was 0.80 ml/min. Detection was carried out by monitoring the absorbance signals at 518 nm. Detector sensitivity was 0.01 Absorbance units full scale (AUFS). For calibration the external standard method was used: the calibration curve was plotted for the malvidin-3-monoglucoside on the basis of peak area. The calibration curve was obtained by injecting 5 solutions (in triplicate) containing increasing concentrations of malvidin-3-monoglucoside. The anthocyanins concentrations were expressed as mg/l of malvidin-3-monoglucoside. The identification and assignation of each compounds was confirmed by high-performance liquid chromatography–electrospray ionisation-mass spectrometry (HPLC–ESI-MS) using a Thermo-Finnigan LCQ Advantage spectrometer (Thermo Finnigan, San Jose, CA) equipped with an electrospray ionisation source and an ion trap mass analyser. The ESI-MS detection was performed in positive mode. The parameters of analysis were: capillary temperature 400 °C, capillary voltage −3 V, nebulizer gas flow 1.75 L min^{-1}, desolvation gas flow 1 L min^{-1}, and spray voltage 5 kV. The analyses were carried out in triplicate.

2.2.4. Trans-resveratrol, catechin, epicatechin and quercetin method

Separation and quantification were carried out by HPLC, as described by Goldberg, Karumankiri, Diamandis, Soleas, and Ng

(1996) with slight modifications. The column used for this separation was an ODS Hypersil column (250 × 4 mm, 5 μm particles diameter) equipped with an ODS Hypersil guard column (20 × 4 mm) (Thermo Quest, Hypersil Division). Samples of 20 μl of wine or calibration standards were directly injected onto the column. Methanol/acetic acid (97.5/2.5) and water/acetic acid (97.5/2.5) were used as the mobile phase (respectively solvent A and B). The elution program was as follows: 0 min, 17.5% A, 82.5% B at a flow-rate of 0.4 ml/min; 5 min, 22.5% A, 77.5% B at a flow-rate of 0.5 ml/min; 30 min, 47.5% A, 52.5% B at 0.5 ml/min until the end of analysis at 50 min. This was followed by a 10-min equilibrium period with the zero-time solvent mixture prior to injection of the next sample. Detection was performed by monitoring the absorbance signals at 306 nm (*trans*-resveratrol), 369 nm (quercetin), and 280 nm epicatechin and catechin). The retention times of the four phenolic compounds, identified by comparison with the pure reference standards, were: catechin (RT = 11.5 min); epicatechin (RT = 17.8 min); *trans*-resveratrol (RT = 34.9 min); quercetin (RT = 46.4 min). Calibration curves obtained by injecting mixed standard solutions containing catechin, epicatechin, *trans*-resveratrol and quercetin were characterised by a correlation coefficient (r^2) >0.998. For each sample of wine, analysis was carried out in triplicate.

2.3. Simple sequence repeats (SSRs) analysis

Genomic DNA was extracted from 0.08 g of young leaf tissue as previously reported from Doyle and Doyle 1987. A set of nine microsatellite primer pairs mapping on 7 of the 19 chromosomes of *V. vinifera* were chosen. In particular, VrZAG7, VrZAG12, VrZAG21, VrZAG47, VrZAG62, VVS2, VVS4, VVIC05 and VVMD27 (Laucou et al., 2011) were used. PCR amplification was carried out in a total volume of 20 μl for each sample containing 0.2 mmol/L dNTPs, 10 mmol/L Tris–HCl (pH 9.0), 0.3 U of AmpliTaq Gold PCR Mastermix (Applied Biosystems, Foster City, California), 0.3 μmol/μl each primer, and 50 ng of template DNA. The PCR cycling conditions were 95 °C for 5 min followed by 40 cycles of 95 °C for 50 s, annealing temperature for 1 min, 72 °C for 50 s, and a final cycle of 72 °C for 7 min. All forward primers were labelled with the fluorescent dyes HEX (Eurofins MWG Operon, Ebersberg, Germany). PCR products were analysed using an ABI 3100 genetic analyser (Applied Biosystems) following the manufacturer's instructions and GeneScan 500-ROX (Applied Biosystems) was used as internal size standard. Data were collected, and allele sizes determined using the Peak Scanner™ v.1.2 software (Applied Biosystems).

2.4. Data analyses

As for chemical analysis data were submitted to multivariate analysis (principal component analysis, PCA) performed using XLSTAT-Pro 7.5.3 software (Addinsoft, Inc., Brooklyn, NY, USA). The least significant difference (LSD) test was used to discriminate among the means of the variables. As for genetic analysis the allele number and frequencies, the expected (He) and observed (Ho) heterozygosity and Polymorphic Index Content (PIC) were calculated using the CERVUS 3.0.3 software (http://www.fieldgenetics.com/pages/home.jsp, Tristan Marshall 1998–2007). The Power of Discrimination (PD) was calculated as follow: $PD = 1 - \sum \sigma p_i^2$ (were p is the frequency of the genotype i). The genetic relationships among genotypes were estimated by the Nei index (Nei & Roychoudhury, 1972) using the GenAlEx 6.41 software (Peakall & Smouse, 2006, http://www.anu.edu.au/BoZo/GenAlEx/). A dendrogram was generated based on the unweighted pair group method of analysis (UPGMA) with the SHAN algorithm through which a genetic distance matrix using R v.2.13.2 (http://www.r-project.org/, Copyright (C) 2011 The R Foundation for Statistical Computing) was obtained. Mantel test was performed as described by Rotondi, Beghè, Fabbri, & Ganino, 2011, using XLSTAT-Pro 7.5.3 software (Addinsoft).

3. Results and discussion

3.1. Biochemical data analysis

The chemical composition of wines is the basis of their quality and authenticity. Specifically, the polyphenolic profile of a given cultivar reflects to a great extent its genetic potential and, therefore, may be used as a tool to differentiate the various cultivars (Guerrero et al., 2009a). Towards this goal we analysed the chemical composition of wines obtained from native (autochthonous) red grape varieties, Aglianico di Taurasi (T), A. del Vulture (V), A. del Taburno (A) and Piedirosso (P) and compared to that of international cultivars, such as Merlot (M) and Cabernet Sauvignon (CS). We also determined the polyphenolic profile from a minor variety, Lingua di Femmina (L), an endemic, uncommon cultivar, found only in Dugenta territory, near city of Benevento. The resulting wine is endowed with not yet characterised sensory properties and could be of interest to winemakers. Finally, the characterisation of such a rare variety has provided the opportunity to recover a cultivar subjected to a constant genetic erosion and reconstitute at least in part the native biodiversity source (heritage).

The chromatic characteristics and total anthocyanin content are reported in Table 1. The color intensity of P, T, V and A wines were similar to CS and M. By contrast, L showed the lowest mean value (4.80). No differences in terms of hue were detected. As for phenols, total anthocyanins mean values for T (559 mg/l), V (535 mg/l) and A (560 mg/l) were similar to CS (533 mg/l), but slightly higher than M (473 mg/l) and P (439 mg/l). L showed substantially less anthocyanin (218 mg/l) than all other wines. Colour intensity and hue are important chromatic characteristics of red wines as they can give a measure of wine colour longevity and appearance (purple or orange for young or old wines, respectively). Given these characteristics, P, T, V and A can be used for long-lasting wines as CS and M; L, instead, cannot be used for this purpose. The high potential in preventing time action of the Aglianico biotypes was also highlighted by their high values of total phenolics (88, 89 and 90 for T, A and V, respectively), as determined by the Folin Ciocalteau Index (FCI). By contrast, due to its low to medium–low values, L has the potential for producing short-lived wine.

The wine colour is due to grape native anthocyanins and the wine pigment derived from them. Grape native anthocyanins profile is also a specific feature of each grape variety and its related wine as well as a measure of its biological value (Revilla et al., 2001). Towards the definition of a phenolic profile for the autochthonous grapevines analysed in our study, a detailed analysis of monomeric anthocyanins was carried out. Malvidin was the most abundant anthocyanin in all the cultivars analysed (Table 1) and its relative abundance showed the following trend: L(77%) > V(70%) > T(68%) > A(66%) > P(60%) > CS(57%) > M(55%).
The most important acylated pigments were malvidin-3 acetylglucoside (Mv-3-ace) and malvidin-3-p-coumarylglucosides (Mv-3-cum), consistently with data from the literature (Garcia-Beneytez, Cabello, & Revilla, 2003). In P, CS, M, and L wines the malvidin concentration distribution was: Mv-3-glc >> Mv-3-ace >> Mv-3-Cum. Conversely, in each variety of Aglianico wine (T, V and A), the concentrations of the three malvidinic forms were distributed as follows: Mv-3-gly >> Mv-3-cum >> Mv-3-ace. Similarly, a higher Mv-3-cum than Mv-3-ace content was also

Table 1
Descriptive statistics calculated on chromatic characteristics, individual anthocyanins[1] and spectrophotometric evaluable phenolics obtained for Piedirosso (P), Cabernet Sauvignon (CS), Merlot (M), Aglianico Taurasi (T), Aglianico Vulture (V), Aglianico Taburno (A) and Lingua di Femmina (L) wines. For each parameter the mean (*x*), the minimum (min) and the maximum (max) values are shown. For each grape cultivar the number of wines analysed is reported in brackets.

Wine		Color intensity (CI)	Hue	Total antroc.[a] (mg/l)	FCI	Tannins (mg/l)	VRF (mg/l)	Dp-3-gly[1]	Cy-3-gly[1]	Pt-3-gly[1]	Pn-3-gly[1]	Mv-3-gly[1]	Mv-3-ace[1]	Mv-3-cum[1]	Mv-3-cum/ Mv-3-ace[1]
P (12)	Min	13.1	0.8	96.7	60.8	3100.0	1178.0	5.0	0.9	5.2	7.5	40.9	4.4	3.8	0.2
	x	15.6 a[2]	0.8 a	439.5 b	71.6 b	3540.0 ab	1639.5 a	13.3 b	16.0 a	28.4 a	21.5 b	202.4 bc	39.9 a	15.0 c	2.7 b
	Max	17.4	0.9	621.5	79.2	3984.0	2351.0	24.7	54.3	70.6	46.4	466.7	139.5	26.0	5.8
CS (8)	Min	14.6	0.7	440.4	55.8	3125.0	1108.0	2.6	0.4	2.5	1.2	21.8	5.1	0.7	0.1
	x	15.5 a	0.8 b	532.6 ab	68.3 c	3580.0 ab	1319.7 c	5.4 b	0.7 (b)	4.8 bc	3.5 c	38.9 cd	11.8 ab	3.2 c	3.7 b
	Max	16.3	0.8	659.8	80.6	4184.0	1640.0	9.1	1.3	8.8	8.4	59.4	19.0	9.4	0.7
M (8)	Min	12.5	0.7	146.0	63.9	2890.0	1189.0	1.1	0.7	1.4	0.9	22.1	9.4	3.1	0.2
	x	14.6 bc	0.8 bc	473.1 ab	70.4 bc	3420.0 b	1538.7 ab	4.3 b	1.0 (b)	5.1 c	4.1 c	38.1 d	12.2 ab	3.9 c	3.1 b
	Max	16.6	0.8	724.5	82.6	3975.0	1734.0	6.7	1.7	7.3	6.9	49.1	16.7	4.4	0.4
T (15)	Min	13.5	0.7	534.5	76.4	3391.0	1124.0	3.5	0.5	4.4	3.0	46.8	3.0	3.4	1.1
	x	14.3 cd	0.7 cd	559.0 a	82.0 a	3710.0 ab	1387.7 bc	11.2 a	12.2 (a)	19.7 a	8.3 bc	164.5 b	5.7 b	18.9 a	0.3 a
	Max	15.0	0.8	578.7	88.4	4218.0	1590.0	18.4	22.0	32.1	13.2	272.2	10.1	41.5	6.6
V (11)	Min	13.6	0.7	271.3	75.7	1849.0	1220.0	3.7	0.0	4.8	3.2	37.1	3.6	4.2	1.1
	x	14.7 bc	0.7 cd	535.5 ab	81.8 a	3560.0 ab	1470.8 b	10.8 a	9.3 (ab)	17.7 ab	7.3 bc	161.3 b	7.0 b	17.8 a	0.4 a
	Max	15.7	0.9	572.3	90.0	3984.0	1700.0	19.5	21.0	31.5	11.8	262.6	12.4	43.4	4.9
A (12)	Min	13.4	0.7	547.8	76.8	3461.0	1230.0	7.4	0.1	9.7	3.7	90.1	4.9	7.6	1.5
	x	13.6 d	0.7 d	559.9 ab	83.1 a	3730.0 a	1439.5 bc	10.5 a	12.9 (a)	18.8 a	9.0 bc	147.7 bc	6.6 b	16.4 ab	0.4 a
	Max	13.8	0.7	581.5	89.0	4175.0	1618.0	17.6	25.4	29.2	15.8	218.5	9.6	24.3	4.5
L (6)	Min	4.2	0.7	185.0	41.0	2480.0	869.0	11.2	0.3	19.6	38.0	104.0	21.0	4.9	0.0
	x	4.8 e	0.7 cd	218.5 c	46.9 d	2740.0 c	1003.1 d	15.5 a	1.0 (b)	29.7 a	54.1 a	466.8 a	32.1 a	9.4 bc	3.4 b
	Max	5.9	0.8	266.0	55.0	2977.0	1105.0	19.3	1.6	38.5	69.9	577.0	44.6	14.4	0.0

[a] Total anthocyanins spectrophotometrically evaluated.
[1] Individual anthocyanins evaluated by HPLC. Dp3gly = delphinidin 3-glucoside, Cy3gly = cyanidin 3-monoglucoside, Pt3gly = petunidin 3-monoglucoside, Pn3gly = peonidin 3-monoglucoside, Mv3gly = malvidin 3-glucoside, Mv3ace = malvidin 3-(6^{II}-acetyl)-glucoside, Mv3cum = malvidin 3-(6^{II}-coumaroyl)-glucoside.
[2] Different letters indicate significant difference among wines in the respective column at $p < 0.05$ according to LSD test.

detected by Castellarin and Di Gaspero (2007) and Mattivi, Guzzon, Vrhovsek, Stefanini, and Velasco (2006) in Aglianico wines, cultivated in Northern Italy. Our results indicated that the influence of latitude of the vineyard and winemaking procedures on the polyphenolic content is negligible for each grape variety examined. The weak relationship we found between the metabolites analysed and the grape geographical origin (and/or current area of cultivation) is consistent with the data reported by Cipriani et al. (2010), who drew similar conclusions analysing 1005 grapevine accessions through an SSR-based genetic approach. Moreover, our results showed that the anthocyanic profile might represent a useful fingerprint of each grape cultivar, confirming earlier findings (Berente et al., 2000). In particular, the acylated anthocyanins are typical of certain grape varieties and have an effective value when linked to the acetylated to *p*-coumaroylated anthocyanins ratio (Revilla et al., 2001).

P, CS, M, T, V, A wines showed similar levels of total tannins, higher than those observed for L (Table 1). Tannins can have different molecular weight, sensory and healthy properties depending on their degree of polymerisation and specific chemical nature (Rinaldi, Gambuti, Moine-Ledoux, & Moio, 2010). It is known that the higher the level of proanthocyanidins, the greater is the perceived astringency (Gambuti, Rinaldi, Pessina, & Moio, 2006). Therefore, wines from L grapes should be less astringent than the others. Although the P, CS, M, T, V and A total tannins maximum values were almost the same, the minimum values were quite different, varying from V (1.8 g/l) to M (2.9) (Table 1). Since the content of these molecules is strongly influenced by viticultural factors (Rodriguez Montealegre, Romero Peces, Chacón Vozmediano, Martinez Gascuena, & García Romero, 2006), oenological practices (Gambuti, Strollo, Erbaggio, Lecce, & Moio, 2007) and wine ageing conditions (Gambuti, Capuano, Lecce, Fragasso, & Moio, 2009), it can be hypothesised that V and M grapes native tannins have a higher sensitivity to these factors than P, CS, T and A.

The value of low-molecular weight phenolics (LMWP), both as monomers and oligomers up to four units (VRF) (Vrhovsek, Mattivi, & Waterhouse, 2001) followed the trend P > CS, M, T, V, A > L (Table 1). LMWP are more bitter than astringent (Brossaud, Cheynier, & Noble, 2008). Therefore, according to our data, P wine should be bitterer than all others. However, as the final wine astringency and bitterness sensations are affected by medium components (e.g. pH, ethanol), a clear assumption of wine sensory characteristics must be based on this additional information.

Regarding the not pigmented low molecular weight wine antioxidant phenols, we determined the levels of *trans*-resveratrol, (+)-catechin, (−)-epicatechin and quercetin of P, CS, M, T, V, A and L as reported in Table 2. Since its discovery in red wine (Siemann & Creasy, 1992), *trans*-resveratrol is one of most investigated phenolic compounds because of the numerous health beneficial effects associated with wine intake and content (Guerrero, Garcia-Parrilla, Puertas, & Cantos-Villar, 2009b). CS and M wines contained the highest levels of *trans*-resveratrol (Table 2), whereas the others showed a wide range of stilbene concentration. Similar values for North Italian Merlot and Cabernet Sauvignon wines (Mattivi, 1993) as well as for Canadian, and Tuscan Cabernet Sauvignon wines (Goldberg et al., 1995) were previously reported. This may be due to the positive effect of a cooler and more humid climate on *trans*-resveratrol synthesis in grapes, as already proposed by Goldberg et al. (1995). Beside genetic and climatic factors, the level of *trans*-resveratrol is strongly affected by factors influencing the hydrolysis of its glycoside (La Torre et al., 2004), by winemaking procedures as well as ageing conditions (Gambuti, Strollo, Ugliano, Lecce, & Moio, 2004). Whether these practices affect the final content of resveratrol in the wines under investigation it is not known so far.

Among wine phenolics, also quercetin, (+)-catechin and (−)-epicatechin exhibit important biological activities and their concentration in grape appears to be strongly dependent on

Table 2
Descriptive statistics calculated on not pigmented low molecular weight antioxidant phenols (mg/l) for Piedirosso (P), Cabernet Sauvignon (CS), Merlot (M), Aglianico Taurasi (T), Aglianico Vulture (V), Aglianico Taburno (A) and Lingua di Femmina (L) wines. For each parameter the mean (x), the minimum (min) and the maximum (max) values are shown. For each grape cultivar the number of wines analysed in reported in brackets.

Wine		*Trans*-resveratrol	(+)-catechin	(−)-epicatechin	Quercetin
P (12)	Min	1.9	69.3	23.5	2.3
	x	4.5 b[1]	182.3 a	75.3 b	7.7 ab
	Max	9.1	457.5	177.0	13.7
CS (8)	Min	1.0	85.8	18.3	1.2
	x	5.9 b	258.1 a	78.8 bc	7.6 b
	Max	12.9	534.6	185.7	20.4
M (8)	Min	0.3	97.3	51.9	5.1
	x	8.5 a	214.6 a	165.8 a	10.6 a
	Max	13.0	421.3	318.3	21.0
T (15)	Min	0.4	7.5	4.0	4.7
	x	1.8 c	69.8 b	52.0 bc	8.0 ab
	Max	3.3	107.5	89.6	12.8
V (11)	Min	0.6	23.4	21.2	4.4
	x	1.9 c	73.8 b	66.9 b	6.8 b
	Max	3.3	168.2	121.0	9.6
A (12)	Min	0.6	17.1	19.1	4.4
	x	2.0 c	65.4 b	48.9 c	8.0 ab
	Max	3.3	107.5	80.7	13.2
L (6)	Min	0.9	45.9	48.4	4.6
	x	2.3 bc	59.3 b	66.2 bc	6.6 ab
	Max	3.5	68.9	79.8	9.4

[1] Different letters indicate significant difference among wines in the respective column at $p < 0.05$ according to LSD test.

genetic factors of each cultivar (Mattivi et al., 2006). In particular, the ratio between catechin and epicatechin is used to discriminate the cultivars employed during winemaking (Goldberg, Karumanchiri, Tsang, & Soleas, 1998). P, CS, M, T, V, A wines showed higher content of (+)-catechin than (−)-epicatechin while L wine had a content of (+)-catechin ⩽(−)-epicatechin. No major differences in quercetin content were detected among the wines analysed, consistent with the data reported in the literature (de Villiers et al., 2005).

To better highlight the important biological entities and reveal insightful patterns from the data obtained, principal component analysis (PCA) was applied to the 72 wines based on the 18 parameters analysed in this work. The first two principal components retained 77.6% of the total variance. Fig. 1B shows the contribution of the variables to the first two axes. The first axis, representing 44.5% of the total variance, was mainly associated with monomeric anthocyanins malvidin-3-glucoside (Mv-3-glc), peonidin-3-glucoside (Pn-3-glc), delphinidin-3-glucoside (Dp-3-glc), petunidin-3-glucoside (Pt-3-glc). The second axis explained the remaining 33.1%, being mainly associated with Mv-3-cum, Cya-3-glc and, to a minor extent with FC Index and Tannins. Three main groups were identified; the first included wines obtained from the L, the second gathered the ones from Aglianico biotypes (V, T, and A), the last included CS and M wines. The second group, partially overlapped the third one for few Aglianico wines with some parameters out of range. Overall, wines were clearly discriminated along the first axis (PC1) based on the cultivar belonging to. M, CS, T, V and A wines located on the left hand of the PC1, indicating high correlation with FCI, total anthocyanins and tannins (Fig. 1B). All Aglianico wines were also well correlated with high values of the Mv-3-cum/Mv-3-ace ratio. The P wines were distributed in the upper side of PCA1 (Fig. 1A). However, when the mean value is taken into consideration, P wines plotted near to the origin of axis (upper-right part), indicating a not well-defined correlation with phenolic parameters (Fig. 1B). L wines were located to the right hand of the PC1 and showed a very low correlation with all phenolic parameters important for long ageing (tannins, total anthocyanins, vanilline reactive flavans and colorant intensity, Fig. 1B). CS, M and L wines were separated along the first axis. Wines obtained from international grape cultivars were discriminated on the basis of high values of ratio cat/epicat and quercetin, while L wines were characterised by high values of Pn-3-gly and Mv-3-ace.

3.2. Molecular data analysis

To efficiently establish the authenticity of the 7 grapevines analysed, we carried out a genetic profiling using SSR markers. A total of 9 pairs of SSR primers were selected for amplification on the basis of high polymorphic and unambiguous profiles. The statistical analysis of SSRs loci is reported in Table 3. A total of 41 alleles were identified in the seven cultivars analysed. Specifically, the number of alleles per locus ranged from 2 (VVS4) to 8 (VrZag21), with an average of 4.6 alleles per locus. The Ho ranged from 0.125 (VrZag7) to 1 (VrZag21 and VVS2), while He ranged from 0.327 (VVIC05) to 0.846 (VrZag21). The PIC assayed within loci ranged from 0.3 (VVIC05) to 0.8 (VrZag21) and most of them (6 out of 9) exceeded the 0.5 threshold value, indicating the high discriminating capacity of the markers used. The Power of Discrimination (PD) values ranged from 0.53 (VVIC05 and VrZag 7) to 0.81 (VrZag21), confirming and supporting the result reported above. By analysing the SSRs loci we identified at least a private (cultivar specific) allele for each cultivar, except for T, although this latter one shared two rare (belonged to two or more cultivars) alleles with V and A at VrZag12 and VrZag21 loci (155 and 190 bp, respectively). Finally, the private/total alleles richness ratio (ARR), clearly indicated a high percentage of selective ability (from 0.20 to 0.67) for each locus, except for VrZag12 and VVS4, which showed a zero value. Altogether, our data indicated that the cultivars analysed show distinct and defined genetic identities. The SSR marker set employed was effective in differentiating cultivars and the results are consistent with previously reported data (Laucou et al., 2011). In order to investigate the power of the markers in discriminating our genetic materials, cluster analysis was carried out on the whole dataset. The dendrogram (Fig. 2) grouped the 7 cultivars into two main clusters: the first included the two international grapevines (M and CS), and the native P and L. Interestingly, this latter was the most genetically distant amongst those belonging to the same cluster. The second cluster contained three cultivars, A, T and V with the first two being genetically closer to each other than to V.

3.3. Comparison of biochemical and genetic results

The cluster obtained by biochemical data was in accordance with the molecular marker dendrogram, although no correlation between Euclidean genetic and biochemical matrixes was found with the Mantel test (p-value = 0.46) (Fig. 1B vs Fig. 2). This suggests that it probably measures a different aspect of the genetic diversity. Similar results have been reported in *Pittosporum undulatum* L. (Mendes et al., 2011) where not superimposable and no correlation between ISSR markers and volatile oils was found. Lack of association between chemical and molecular data has been also reported in *Juniperus brevifolia* (Seub.) (Lima, Trindade, Figueiredo, Barroso, & Pedro 2010), and *Thymus caespititius* Brot. (Trindade et al. 2009). However, when we selected a subset of chemical parameters, namely those related to the phenylpropanoid pathway (CI, anthocyanin, FCI, *trans*-resveratrol, (+)-catechin, (−)-epicatechin, (+)-catechin/(−)-epicatechin, Pn-3-glc, Mv-3-cum, Mv-3-ace/Mv-3-cum) a statistically significant correlation was found between datasets (Mantel results, $r = 0.815$, $p \leqslant 0.03$). Among the metabolites related to phenylpropanoids pathway, only quercetin showed no correlation between datasets. Indeed, quercetin is involved in numerous copigmentation reactions during winemaking

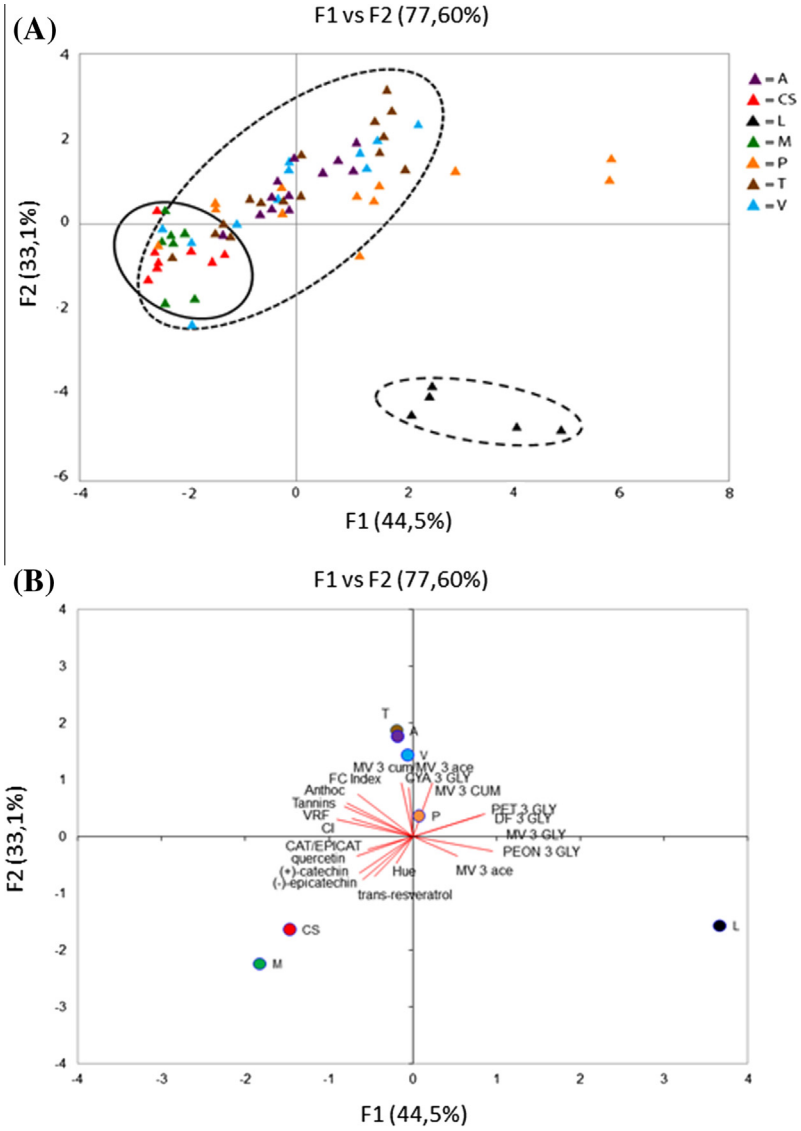

Fig. 1. (A) Principal component analysis generated from all biochemical parameters for 72 wines analysed. (B) Bi-plot PCA reporting the mean position of each cultivar and the components that drive them to the positioning.

Table 3
Results of SSRs statistical analysis obtained using nine microsatellite markers on Piedirosso (P), Cabernet Sauvignon (CS), Merlot (M), Aglianico Taurasi (T), Aglianico Vulture (V), Aglianico Taburno (A) and Lingua di Femmina (L) grapevines. For each locus the number of total and private (grapevine-specific) alleles, their size range (bp), and some genetic statistics (for details see Section 2) were reported.

Locus	No alleles for locus	Ho	He	PIC	PD	Allele size range (bp)	No private alleles	ARR
VrZAG7	3	0.13	0.47	0.40	0.53	106–152	1	0.33
VrZAG12	5	0.88	0.81	0.76	0.75	106–163	0	0.00
VrZAG21	8	1.00	0.85	0.81	0.81	105–214	3	0.38
VrZAG47	5	0.88	0.62	0.56	0.69	152–170	3	0.60
VrZAG62	4	0.50	0.59	0.51	0.75	183–200	2	0.50
VVIC05	3	0.38	0.33	0.29	0.53	156–160	1	0.33
VVMD27	6	0.88	0.69	0.63	0.69	173–191	4	0.67
VVS2	5	1.00	0.73	0.67	0.75	130–155	1	0.20
VVS4	2	0.50	0.48	0.36	0.59	167–174	0	0.00
Mean	4.56	0.68	0.62	0.55	0.68	105–215	2	0.33

and wine ageing (Lambert, 2011). Until now numerous studies have showed the relationship between phenylpropanoid metabolites in grapes and the expression of genes controlling their biosynthetic pathway (Castellarin & Di Gaspero, 2007). However, no correlation between non-coding DNA fragments (SSR markers) and metabolic dataset in grape has been provided so far. Previously, Navarro et al., 2007, demonstrated that the total contents of antocyanic compounds and *trans*-resveratrol in grape berries were closely related to genetic characteristics and highly suitable to distinguish grapes cultivars or to authenticate monovarietal wines. In *Olea europea* L., following a similar approach, Rotondi et al. (2011) found a significant correlation between

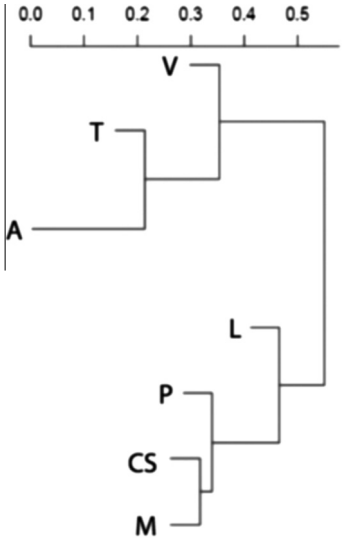

Fig. 2. UPGMA dendrogram of SSR data of Piedirosso (P), Cabernet Sauvignon (CS), Merlot (M), Aglianico Taurasi (T), Aglianico Vulture (V), Aglianico Taburno (A) and Lingua di Femmina (L) grapevines. The similarity on the *x*-axis is based on the Nei's coefficient.

selected fatty acids and genetic matrixes by analysing nine olive cultivars and the olive oils they derived from.

4. Conclusion

In conclusion, two major points can be drawn from our studies. First, the diverse grape cultivars show enough distinct phenolic profiles to allow a statistical differentiation. Second, the SSR-based data and the profile of the phenylpropanoid molecules selected in this work exhibited a high correlation. As far as we know, this is the first report that correlates non-coding DNA fragments (SSR markers) with metabolic dataset in *V. vinifera* L. Therefore, we believe that our approach might be reasonably adopted in the future for characterisation and traceability purposes of grapevines and corresponding wines. Further analysis will help to verify whether winemaking procedures, as well as ageing conditions may influence the correlation between SSR-based data and polyphenolic compounds. In addition it would be interesting to investigate if other metabolites, different from phenylpropanoids, might correlate with the molecular markers.

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