

Intra-varietal Agronomical Variability in *Vitis vinifera* L. cv. Cannonau Investigated by Fluorescence, Texture and Colorimetric Analysis

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Submission for publication: November 2015

Accepted for publication: February 2016

Keywords: Biotypes, anthocyanins, CIELab, FERARI, skin thickness

To date, innovative, rapid and non-invasive techniques have been used to assess variations in morphological and technological characteristics between grape cultivars. In order to improve knowledge of the qualitative expression of the genetic traits of cv. Cannonau (syn. Grenache), fluorescence, texture and colorimetric analyses were performed on 85 biotypes attributable to this variety. Eighty-five Cannonau biotypes cultivated in the same vineyard were analysed in order to evaluate their morphological and agronomical characteristics. As regards the morphological traits, of the six descriptors observed, five were able to discriminate the Cannonau population according to different expression levels. Must composition, berry colour (using the Minolta tristimulus colorimeter), texture (assessed using the TaxT2i plus texturimeter) and fluorescence (using the Multiplex III detector) were determined on clusters harvested at the same time. Cluster analysis was able to separate the Cannonau population into five groups, characterised by differences in the following criteria: L*, a* and b*, berry skin thickness, FERARI, TSS, pH and titratable acidity, total polyphenols and total anthocyanins. The FERARI index was highly and positively correlated with total anthocyanins and negatively correlated with grape colour, while berry weight and berry skin thickness were not correlated with any variable assessed. The remarkable intra-variability encountered for the qualitative and quantitative characters in the Cannonau variety suggests that new biotypes could be selected for different oenological purposes, using techniques such as the measure of fluorescence and colorimetry. This is the first study carried out within the cv. Cannonau using innovative and classical techniques suitable to investigate intra-varietal variability.

INTRODUCTION

Natural biodiversity has played a leading role in the evolution of agriculture over the centuries, in terms of which crops were selected according to the identification, manipulation and domestication of wild species (Chess & Nieddu, 2005). Although a cultivar consists of vines presenting the same basic characteristics, there is remarkable genetic variability within each cultivar, resulting from the occurrence of spontaneous genetic mutations and their transmission to subsequent progeny (Mannini *et al.*, 2011). Vegetative propagation helped to multiply and spread cultivars and clones around the world, further expanding intra-varietal variability. Numerous studies investigating the impact of clonal variability on the yield component and fruit composition of different grapevine varieties have been carried out since the late 1980s. Knowledge about intra-varietal variability is widely recognised in the wine industries. Indeed, understanding the agronomic and technological characteristics of the different clones and their wines has led to an increase in oenological

products and improvements in wine quality. Differences among clones may relate to amphelographic characteristics and phenological phases (Nieddu *et al.*, 2006), disease resistance (Van Leeuwen *et al.*, 2012), yield and berry composition (Fidelibus *et al.*, 2006; Anderson *et al.*, 2008; Mercenaro *et al.*, 2009), as well as secondary metabolite concentrations (Belancic & Agosin, 2007; Mulas *et al.*, 2011). However, the level of intra-varietal variability is also cultivar dependent. Some cultivars, such as Pinot noir and Chenin, show great variability, whereas others, such as Cabernet Sauvignon and Garnacha Tintorea, are more homogeneous (Boso *et al.*, 2004). Another approach to studying the intra-varietal variability in *Vitis vinifera* L. involves molecular techniques (Carimi *et al.*, 2011). Meneghetti *et al.* (2011) studied 53 Garnacha (syn. Grenache) accessions coming from France, Spain and Italy using a PCR-derived marker system to characterise the clones. They confirmed that all accessions belonged to the same cultivar, yet the molecular marker system was clearly able to distinguish the Garnacha

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Acknowledgements: This work was performed within the project SQFVS (APQ P6) and carried out by the Convisar wineries: Argiolas, Serdiana; Cantina Gallura, Tempio; Cantina Li Duni, Badesi; Cantina delle Vigne di Piero Mancini, Olbia; Cantina del Mandrolisai, Sorgono; Cantina Trexenta, Senorbi

accessions according to their area of origin. The authors revealed high levels of genetic similarity among the Spanish and French accessions, and high levels of genetic dissimilarity among the Italian accessions originating from different zones of cultivation (Alicante, Tocai Rosso, Gamay Perugino, Cannonau).

The Garnacha grapevine, covering 185 000 hectares worldwide, is one of the most widely cultivated wine grapes in the world (Anderson, 2013). The European wine growers know it by different names, depending on their geographical origin: Garnacha Tinta and Grenache Noir are the Spanish and French names respectively, while in Italy this grape is known as Tocai Rosso (north-east regions), Alicante, Gamay Perugino (central regions) and Cannonau (on the island of Sardinia).

Anthocyanins, mainly contained within the grape skin, are the key compounds responsible for the colour of red grapes and of their wines; Cannonau is characterised, and can easily be distinguished from other cultivars, for its low anthocyanin content and high percentage of malvidin, which accounts for about 66% of the colour fraction (Vacca *et al.*, 2009; Fernandes de Oliveira *et al.*, 2015).

An innovative non-invasive tool for testing grape phenolic maturity and for establishing the ideal harvest time is the portable fluorescence detector, the Multiplex (FORCE-A, Orsay, France) (Cerovic *et al.*, 2008; Ghozlen *et al.*, 2010a; 2010b). At present, to the best of our knowledge, only one study has dealt with the use of the Multiplex for determining colour differences among clones of table grapes (Bahar *et al.*, 2012). The results of this study demonstrated the efficient use of this instrument for the analysis of grape quality.

Similarly, variability in grape skin colour can be assessed using the CIELab system by tristimulus colorimetry, allowing different varieties to be compared (Rolle & Guidoni, 2007) and grape colour to be correlated with anthocyanin content (Liang *et al.*, 2011).

Grape skin hardness, on the other hand, evaluated by the skin break force and skin break energy parameters, is a mechanical property, as is skin thickness, considered appropriate for the estimation of skin cell wall degradability and, therefore, for the extractability of anthocyanins from the berry skins into the must/wine (Río Segade *et al.*, 2008; Rolle *et al.*, 2011).

To date, neither the fluorescence technique using the Multiplex nor colour evaluation using CIELAB have been used to discriminate biotypes within the same wine grape variety. The purpose of this manuscript was to evaluate the intra-varietal level of variability of 85 biotypes of cv. Cannonau, selected during a previous massal genetic improvement programme. For this reason, agronomical measurements on vines, chemical analyses on must, innovative techniques such as fluorescence, texture and colorimetric analysis on berries were used. All the biotypes, coming from different areas of Sardinia, were planted in the same vineyard and subjected to the same agronomical practices. The results of the combined application of the Multiplex portable fluorescence detector, the CIELab colorimetric system and instrumental texture analysis of wine grapes, are reported and discussed here.

MATERIALS AND METHODS

Sampling

The trial was conducted in an experimental vineyard planted in 2009 and located in the Parteolla Valley of Serdiana in south-west Sardinia, Italy. The soil is alluvial, homogeneous, and has an average depth of 80 to 100 cm, with the following characteristics: sand 51%, clay 24.9% and silt 24.1%; it has a pH of 7.44 and the organic matter content is 16 g/Kg. The vineyard covers 2 500 m²; a total of 103 biotypes of cv. Cannonau (a.k.a. Grenache) are present in this plot, with 11 contiguous vines per each biotype. The vines were grafted onto 1103 P rootstock and spaced 2.1 m x 1.0 m apart. The plants were trained according to the vertical shoot position in a cane-pruned system with a total of eight buds per vine. For the scope of the present study, 85 biotypes were assessed. No cluster thinning was performed in order to evaluate the real yield and, consequently, the oenological potential of these biotypes. From the pea-size stage to véraison the vines received a total of three irrigations by means of a drip irrigation system (corresponding to about 700 m³ ha⁻¹ year⁻¹). Weed control along the rows was performed using herbicide. The experiment started during the commercial harvest period, with all grapes being hand-picked on 2012-10-01. For each selection under observation, nine of 11 vines were harvested and the yields recorded for each plant. To define yield components, cluster and berry weights were recorded by weighing 18 clusters randomly chosen from each plot, at two clusters per vine, with 20 berries randomly chosen from each cluster. Practically, agronomical data for each selection were represented by the mean of 18 biological replications.

Fruit composition

For the assessment of the fruit composition, the nine vines were divided into three groups, each containing three contiguous vines. A total of 0.5 kg of berries, with pedicel, and randomly picked, were collected from each group. The berries were weighed and crushed and the juice was analysed for sugar content, expressed as total soluble solids (TSS) (°Brix), pH and titratable acidity according to the methods of the Office International de la Vigne et du Vin (OIV, 1990). The amounts of total polyphenols and total anthocyanins were evaluated by spectrophotometry, according to Di Stefano and Cravero (1991), with ultraviolet absorption measured at 700 nm and 520 nm. Chemical parameters were represented by the mean of the three biological replicates.

Morphological data

At the time of harvest, morphological data were collected for all selections using six primary descriptors from the OIV Grape Descriptor List (OIV, 2001). In particular, three descriptors described cluster characteristics: density (OIV code 204), shape (OIV code 208) and number of wings of the primary cluster (OIV code 209); and three descriptors identified berry characteristics: uniformity of size (OIV code 222), shape (OIV code 223) and skin colour (OIV code 225). During the dormant season, the weight of the pruning wood and the number of shoots per vine were recorded in order to estimate vegetative growth. The weight of the pruning wood was also used to calculate the Ravaz Index (yield-to-pruning-weight ratio).

Colour analysis

Ten berries per sample were analysed using a Minolta tristimulus colorimeter CR 300 (Osaka, Japan), with D65 as illuminant and a 2° standard observer. CIELab space colour was used, where L* indicates lightness, a* indicates red (+ a) to green (- a) colours, and b* indicates yellow (+ b) to blue (- b) colours. Hue angle ($\tan^{-1}b^*/a^*$) and chroma ($a^2+b^2)^{0.5}$ parameters were also calculated. The colorimeter was calibrated using a standard white plate.

Fluorescence analysis

The same berries were placed on a square dark tray with 6 cm sides and analysed using the portable fluorescence detector Multiplex III (Force A, Orsay, France) (Ghazlen *et al.*, 2010a), as reported in the literature, where it was used to indicate the ripeness grade of table grapes (Bahar *et al.*, 2012). Each berry was analysed twice on each side, thus obtaining four measurements per sample. The following parameters were determined: BRR_FRF (blue to red fluorescence), FER_RUV (fluorescence excitation), FLAV (log of FER_RUV), FER_RG (fluorescence excitation ratio), FER_RB, ANTH_RG (log (FRF_R/FRF_G)), ANTH_RB and FERARI (log of FRF_R).

Texture analysis

Berries collected at harvest were frozen at -20°C until analysis. In order to preserve the berry integrity, the samples were stored with the pedicel and subjected to fast-freezing to avoid the development of micro ice crystals that could interfere with the skin separation from the berry. Texture analysis was performed using a TA.XT2i plus texturimeter (Stable Micro System, Surrey, UK) equipped with a HDP/90 platform and a load cell of 5 kg, calibrated for 2 kg of force for berry skin thickness, and a load cell of 30 kg, calibrated for 10 kg of force for seed deformation. Berry skin thickness was performed on 20 berries for each clone, using a small lancet and a clamp to separate a piece of skin from the lateral side of each berry. A sample of 0.25 cm⁻¹ from each berry separated from the pulp was prepared as reported in Río-Segade *et al.* (2008). To evaluate berry skin thickness (Sp_{sk}), an instrumental trigger threshold equal to 0.05 N was inserted to permit the plane surface probe to adhere to the skin sample before the acquisition started (Battista *et al.*, 2015). Before starting the test, the instrument was calibrated for force and distance. The mechanical properties of seeds were evaluated as follows – seeds were withdrawn from 10 berries, dried with adsorbent paper and analysed for the following parameters: seed break force (N, as F_s) at the moment of compression (50% deformation); seed break energy (mJ, as W_s); seed

break distance (mm); and seed deformation index (%), as DI_s , calculated as previously reported (Letaief *et al.*, 2008; Rolle *et al.*, 2012). All the setting parameters are reported in Table 1.

Statistical analysis

The statistical analysis was performed using Statistica 6.0 for Windows (Statsoft). The multivariate statistical techniques used were principal component analysis, to investigate the relationships between the variables and the 85 biotypes, and cluster analysis, to identify clusters on the basis of similarities among the biotypes, using complete linkages between groups and the square Euclidean distance. One-way Anova, followed by LSD Fisher's test, was applied to estimate significant differences among the groups obtained from the cluster analysis. Pearson's correlation was used to investigate the relationships between the measured parameters.

RESULTS

Regarding the ampelographic traits, only one descriptor was unable to discriminate the Cannonau population according to different expression levels: all biotypes were characterised by the OIV code number 223 berry shape, indicating an ellipsoid form. With reference to OIV code number 222 (Table 2), 92% of the population exhibited berries with a uniform size, while only 8% of the biotypes showed non-uniform berry sizes. The berry skin colour descriptor, OIV code number 225, grouped the biotypes into four distinct expression levels. More specifically, visual analysis assigned 11% of the biotypes to a rose colour, 14% to a red colour, and 48% and 27% exhibited dark red violet and blue black skin colours respectively. With regard to the grape cluster descriptors, three levels of cluster density (OIV code number 204) were recorded. Forty-three biotypes were characterised as dense clusters, while 33 were catalogued as very dense clusters. Only nine biotypes were identified to be of medium cluster density. Regarding the cluster shape (OIV code number 208), the conical shape was detected most frequently. Cylindrical and funnel shaped clusters were found in 24 and 19 of the 85 biotypes respectively. Another descriptor used to discriminate between biotypes was the number of wings of the primary cluster (OIV code number 209). Of the 85 biotypes analysed, 70 presented one or two wings, 14 presented three or four wings, and wings were absent in just four biotypes. With regard to yield, biotype B46 exhibited the lowest production, at 0.99 kg/vine, while the highest production was recorded for B10, at 4.88 kg/vine. The average yield for the whole population was 2.39 kg/vine.

TABLE 1

Texture analysis settings for skin thickness and seed hardness determination using a TA.XT2i plus texturimeter.

Test	Probe	Test speed	Deformation	Mechanical property
Skin thickness	sms p2, ø 2 mm	0.20 mm/sec	–	Sp_{sk} (µm) F_s seed break force (N) W_s seed break energy (mJ)
Seed hardness	sms p35, ø 35 mm	1 mm/sec	50%	Seed break distance (mm) Seed deformation index (%)

TABLE 2

Ampelographic descriptor expression levels of the Cannonau biotypes. Cluster characteristics are related to density (OIV 204), shape (OIV 208) and number of wings of the primary cluster (OIV 209); berry characteristics are related to uniformity of size within the cluster (OIV 222) and skin colour (OIV 225).

Biotypes	(OIV 204)	(OIV 208)	(OIV 209)	(OIV 222)	(OIV 225)
BT 1	medium	conical	1 - 2 wings	uniform	blue-black
BT 2	medium	conical	1 - 2 wings	uniform	blue-black
BT 3	dense	conical	3 - 4 wings	uniform	dark red-violet
BT 4	dense	cylindrical	1 - 2 wings	uniform	rose
BT 5	dense	cylindrical	1 - 2 wings	uniform	rose
BT 6	medium	conical	1 - 2 wings	uniform	dark red-violet
BT 7	dense	conical	1 - 2 wings	uniform	red
BT 8	dense	funnel shaped	1 - 2 wings	uniform	blue-black
BT 9	dense	funnel shaped	1 - 2 wings	uniform	dark red-violet
BT 10	very dense	funnel shaped	1 - 2 wings	uniform	rose
BT 11	dense	conical	1 - 2 wings	uniform	blue-black
BT 12	dense	cylindrical	1 - 2 wings	uniform	dark red-violet
BT 13	very dense	funnel shaped	1 - 2 wings	uniform	blue-black
BT 14	dense	conical	3 - 4 wings	non uniform	red
BT 15	dense	conical	absent	uniform	dark red-violet
BT 16	dense	cylindrical	1 - 2 wings	uniform	dark red-violet
BT 17	very dense	conical	1 - 2 wings	uniform	red
BT 18	dense	conical	3 - 4 wings	uniform	red
BT 19	dense	conical	1 - 2 wings	uniform	rose
BT 20	dense	cylindrical	1 - 2 wings	uniform	rose
BT 21	very dense	cylindrical	absent	uniform	rose
BT 22	dense	funnel shaped	1 - 2 wings	non uniform	blue-black
BT 23	dense	funnel shaped	1 - 2 wings	non uniform	dark red-violet
BT 24	very dense	cylindrical	1 - 2 wings	uniform	red
BT 25	dense	conical	1 - 2 wings	uniform	red
BT 26	very dense	conical	3 - 4 wings	uniform	dark red-violet
BT 27	dense	conical	1 - 2 wings	non uniform	blue-black
BT 28	dense	conical	1 - 2 wings	non uniform	rose
BT 29	dense	conical	1 - 2 wings	uniform	red
BT 30	dense	cylindrical	1 - 2 wings	non uniform	red
BT 31	dense	cylindrical	3 - 4 wings	uniform	dark red-violet
BT 32	dense	conical	1 - 2 wings	uniform	dark red-violet
BT 33	very dense	funnel shaped	1 - 2 wings	uniform	dark red-violet
BT 34	very dense	funnel shaped	1 - 2 wings	uniform	dark red-violet
BT 35	very dense	cylindrical	absent	uniform	dark red-violet
BT 36	dense	cylindrical	1 - 2 wings	uniform	dark red-violet
BT 37	dense	conical	1 - 2 wings	uniform	dark red-violet
BT 38	very dense	funnel shaped	1 - 2 wings	uniform	dark red-violet
BT 39	medium	conical	1 - 2 wings	uniform	dark red-violet
BT 40	very dense	cylindrical	1 - 2 wings	uniform	dark red-violet
BT 41	very dense	cylindrical	1 - 2 wings	uniform	dark red-violet
BT 42	dense	conical	1 - 2 wings	non uniform	dark red-violet
BT 43	very dense	cylindrical	1 - 2 wings	uniform	red
BT 44	dense	cylindrical	1 - 2 wings	uniform	rose
BT 45	very dense	cylindrical	1 - 2 wings	uniform	red
BT 46	very dense	cylindrical	1 - 2 wings	uniform	red
BT 47	very dense	funnel shaped	1 - 2 wings	uniform	blue-black
BT 48	dense	funnel shaped	1 - 2 wings	uniform	dark red-violet
BT 49	very dense	funnel shaped	1 - 2 wings	uniform	blue-black
BT 50	dense	conical	1 - 2 wings	uniform	blue-black
BT 51	dense	conical	3 - 4 wings	uniform	dark red-violet
BT 52	very dense	conical	1 - 2 wings	uniform	blue-black
BT 53	dense	conical	1 - 2 wings	uniform	dark red-violet
BT 54	very dense	cylindrical	1 - 2 wings	uniform	red
BT 55	dense	funnel shaped	1 - 2 wings	uniform	rose
BT 56	very dense	funnel shaped	1 - 2 wings	uniform	dark red-violet
BT 57	medium	conical	3 - 4 wings	uniform	dark red-violet
BT 58	very dense	conical	1 - 2 wings	uniform	blue-black

TABLE 2 (CONTINUED)

Biotypes	(OIV 204)	(OIV 208)	(OIV 209)	(OIV 222)	(OIV 225)
BT 59	very dense	cylindrical	1 - 2 wings	uniform	dark red-violet
BT 60	dense	cylindrical	1 - 2 wings	uniform	blue-black
BT 61	very dense	conical	1 - 2 wings	uniform	dark red-violet
BT 62	medium	conical	3 - 4 wings	uniform	dark red-violet
BT 63	very dense	conical	1 - 2 wings	uniform	dark red-violet
BT 64	dense	funnel shaped	1 - 2 wings	uniform	blue-black
BT 65	medium	conical	1 - 2 wings	uniform	blue-black
BT 66	very dense	cylindrical	1 - 2 wings	uniform	blue-black
BT 67	very dense	funnel shaped	3 - 4 wings	uniform	dark red-violet
BT 68	very dense	conical	1 - 2 wings	uniform	dark red-violet
BT 69	dense	conical	1 - 2 wings	uniform	dark red-violet
BT 70	very dense	conical	1 - 2 wings	uniform	dark red-violet
BT 71	medium	cylindrical	1 - 2 wings	uniform	dark red-violet
BT 72	very dense	conical	1 - 2 wings	uniform	blue-black
BT 73	very dense	conical	1 - 2 wings	uniform	dark red-violet
BT 74	dense	funnel shaped	3 - 4 wings	uniform	dark red-violet
BT 75	very dense	conical	1 - 2 wings	uniform	blue-black
BT 76	very dense	conical	1 - 2 wings	uniform	blue-black
BT 77	dense	conical	1 - 2 wings	uniform	dark red-violet
BT 78	dense	cylindrical	1 - 2 wings	uniform	blue-black
BT 79	dense	funnel shaped	1 - 2 wings	uniform	blue-black
BT 80	dense	conical	3 - 4 wings	uniform	dark red-violet
BT 81	dense	conical	1 - 2 wings	uniform	dark red-violet
BT 82	very dense	funnel shaped	1 - 2 wings	uniform	blue-black
BT 83	medium	conical	3 - 4 wings	uniform	dark red-violet
BT 84	dense	conical	1 - 2 wings	uniform	dark red-violet
BT 85	dense	cylindrical	1 - 2 wings	uniform	blue-black

Regarding the yield component analysis, berry weights ranged between a minimum of 1.44 grams and a maximum of 2.51 grams, detected on biotypes B44 and B8 respectively. The overall mean berry weight was 1.96 grams. Concerning the cluster parameters, the distribution of the number of clusters per vine among the biotypes varied between five and 15, while cluster weight varied between 160 grams (biotype B31) and 614.1 grams (biotype B18). The average number of clusters per vine and the cluster weight for the whole population was 9 and 293.7 kg respectively. According to the coefficient of variation index, berry weight showed the least variable yield component ($CV = 0.11$), whereas similar values were calculated for the number of clusters per vine and cluster weight (0.24 and 0.23 respectively) (Fig. 1).

As far as the data of fluorescence, texture and colorimetric analysis, a huge variability within the population of 85 Cannonau biotypes was observed for all the parameters assessed. More specifically, the FERARI index varied between 1.43 and 2.39, with an average of 2.10. The texture parameter, berry skin thickness, was characterised by a population average of 90 μm and a CV of 16.2%. At last, a noteworthy variability also was detected for the colorimetric coordinates L^* , a^* and b^* , which ranged between 23.92 and 37.39, 2.15 and 11.12, and -4.97 and 9.42 respectively.

The results of the principal component analysis (PCA) performed to establish the relationships between the variables and the 85 biotypes are reported in Fig. 2. The first two principal components explained 62% of the variability in the original data. In the PCA analysis, all variables were initially considered, but the best performance

of the biotypes' discrimination analysis was obtained when using 11 of them. The variables that best separated the biotypes were the following: L^* , a^* and b^* CIELab coordinates (colorimetric analysis), berry skin thickness (texture analysis), FERARI (fluorescence analysis), TSS, pH and titratable acidity (technological maturity), total polyphenols and total anthocyanins (phenolic maturity), and berry weight (agronomic parameter). Eight biotypes were characterised as being completely distinct from the others (Fig. 2a). The first component, explaining 46.83% of the variance, was associated with the CIELab coordinates and FERARI, pH, TSS and anthocyanins, while the second component was correlated with berry skin thickness, acidity, total polyphenols and berry weight (15.16% of the variance). The eight biotypes identified as being highly distinct from all others were characterised by high CIELab coordinate values and titratable acidity, and low values of FERARI and TSS (Fig. 2b).

The 11 variables used to perform the PCA analysis were used in order to enhance the differentiation between the 85 biotypes to perform a cluster analysis. The dendrogram obtained using the complete linkage and the square Euclidean distance is reported in Fig. 3. Five groups were identified by cluster analysis. The first two groups (named 1 and 2) include the eight biotypes selected by PCA. Group 1 contains five biotypes and group 2 contains three biotypes. Groups 3, 4 and 5 are composed as follows: group 3 includes 34 biotypes, group 4 31 biotypes and group 5 12 biotypes. These last three groups are characterised by low CIELab coordinate values, high values of total polyphenols and

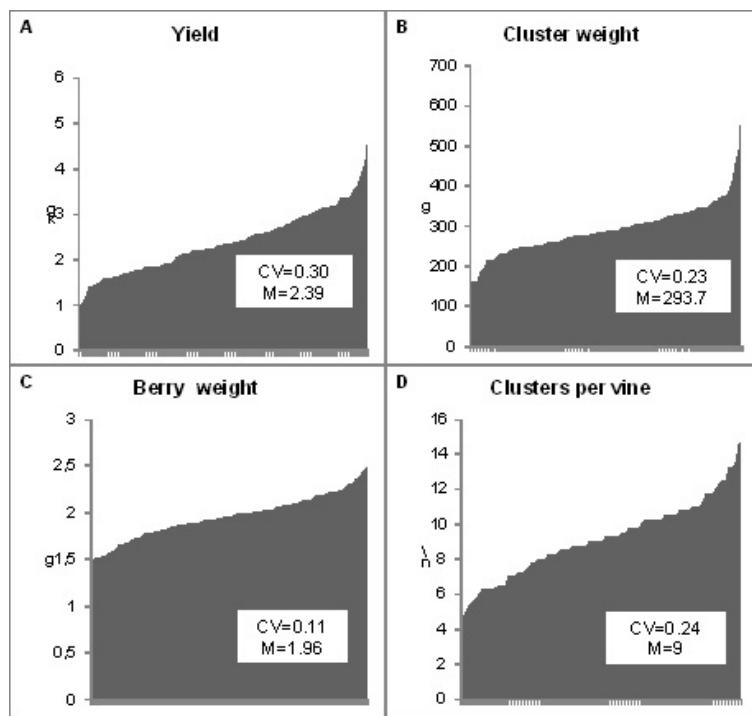


FIGURE 1

Distribution of yield (A) and its components, cluster weight (B), berry weight (C) and number of clusters per vine (D) in the Cannonau population. Coefficient of variation (CV) and mean (M) are reported.

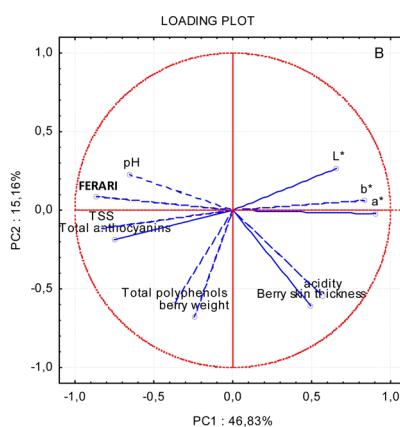
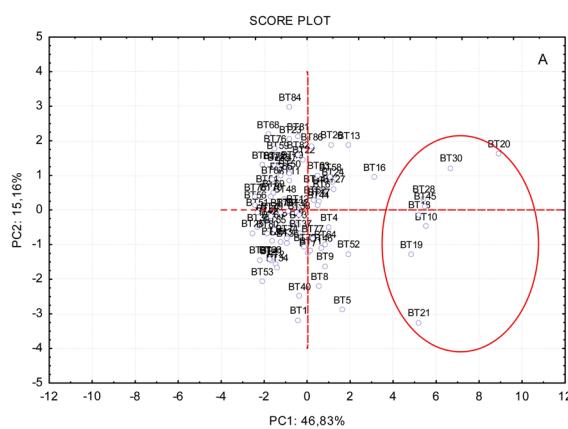


FIGURE 2

PCA score plot (a) and loading plot (b) of the 85 Cannonau biotypes, performed using the variables best able to separate the 85 biotypes. a) BT means biotype, b) TSS means total soluble solids, L* indicates lightness, a* indicates red (+ a) to green (- a) colours, and b* indicates yellow (+ b) to blue (- b) colours.

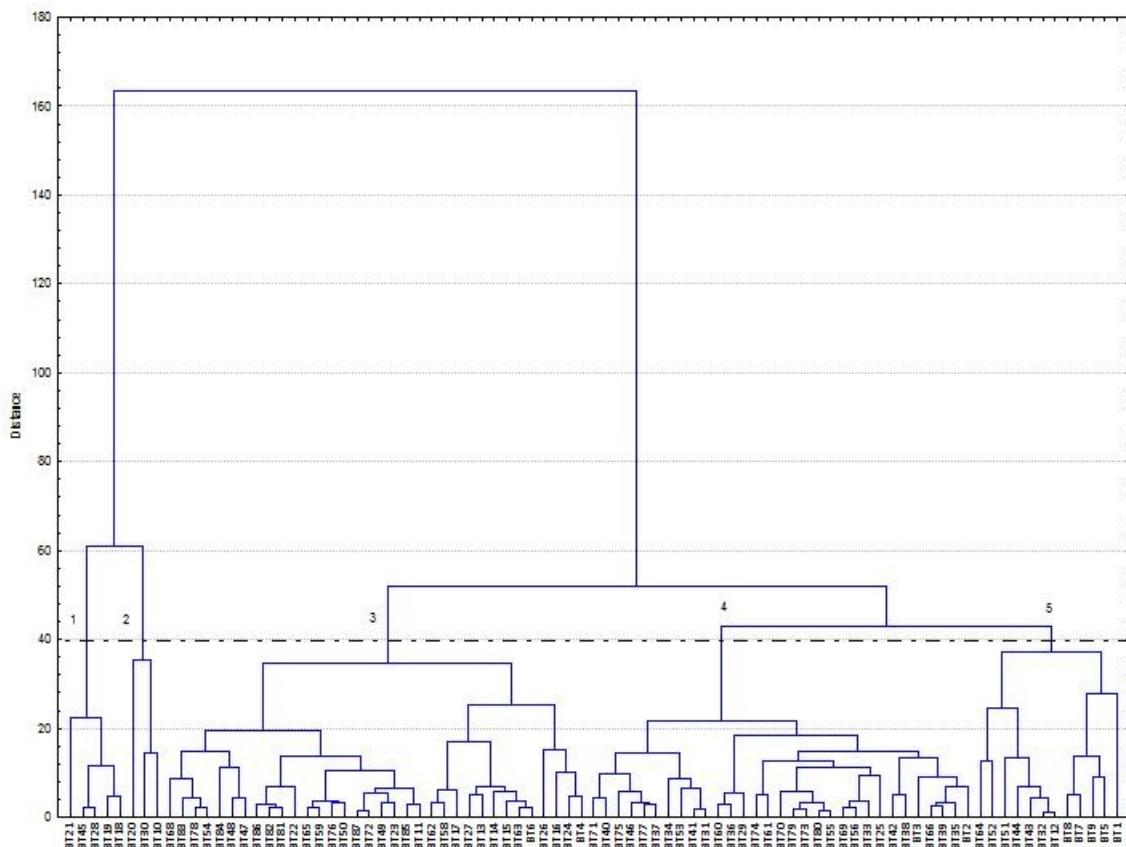


FIGURE 3

Cluster analysis of the 85 Cannonau biotypes performed with the same variables used for PCA analysis. BT means biotype.

anthocyanins, FERARI and TSS, and low values of berry skin thickness and titratable acidity. Fig. 4 summarises the differences between the different groups in relation to the colorimetric parameters (CIELab). As can be observed, the first two groups were characterised by higher values of L^* , a^* and b^* colour parameters, while in groups 3, 4 and 5, the b^* parameter showed negative values. Fig. 5 reports the profiles of the five groups obtained using the normalised mean values of the following variables: berry skin thickness, total polyphenols, total anthocyanins, FERARI, berry weight, TSS, pH and titratable acidity. The biotypes belonging to groups 4 and 5 are richer in anthocyanins and polyphenols than the other groups, while groups 1 and 2 are characterised by a low content of total anthocyanins, low values of FERARI and a low TSS content. Table 3 reports the mean, standard deviation (SD) and coefficient of variation (CV %) of each variable for each group, and the significant differences among the groups for each variable according to LSD Fisher's test.

Table 4 reports the Pearson's correlation coefficients ($P < 0.05$), which quantify the relationships between the considered variables. Berry skin thickness and berry weight were not correlated with any other variable, and production per hectare was highly and positively correlated with the L^* and b^* colour parameters and with titratable acidity. The FERARI index results were highly correlated with total anthocyanins, and also positively correlated with technological maturity (pH and titratable acidity); on the other hand, this index's results were negatively correlated

with grape colour (CIELab system).

DISCUSSION

The present study characterised 85 Cannonau biotypes according to their ampelographic, agronomical and technological traits. The different biotypes were selected from various sites across Sardinia and then grafted and cultivated in the same vineyard in south-west Sardinia. High levels of variability were revealed for almost all of the parameters studied. For instance, five of the six primary morphological descriptors specified on the OIV Grape Descriptor List (OIV, 2001), which is used to describe grapevine varieties, were able to distinguish between the different biotypes within the cultivar.

However, the aim of the present study was to assess the variability within the Cannonau population also using new, fast and innovative techniques never used before now to describe biotypes. This new approach, which comprised non-invasive techniques such as instrumental texture analysis, fluorescence analysis and colorimetric analysis, was successfully shown to be able to separate the biotypes into distinct groups, highlighting the possibility of being an additional evaluation tool in the projects of genetic improvement in winemaking.

There are many papers in the literature dealing with the use of CIELab parameters for red wine grape evaluation during the ripening process (Rolle & Guidoni, 2007) and for wine colour evaluation (Gil-Muñoz *et al.*, 1997; Pérez-Caballero *et al.*, 2003); yet, to our knowledge, no data are

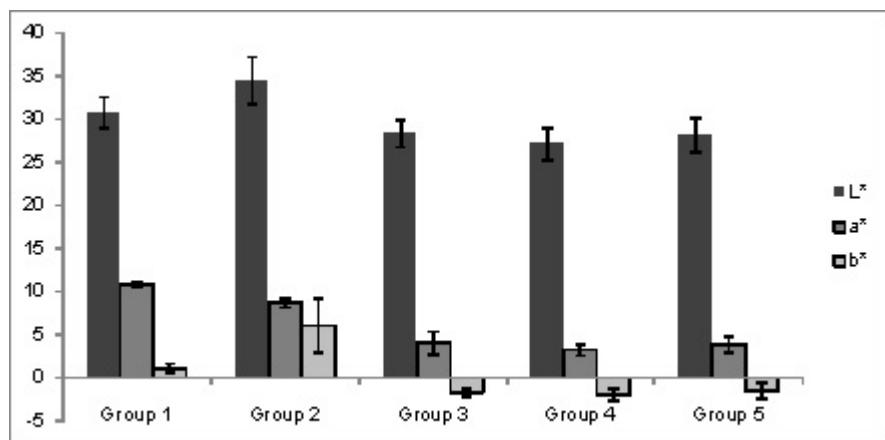


FIGURE 4

L^* , a^* and b^* CIELab parameters, obtained by tristimulus colorimetry, for the biotype groups obtained by cluster analysis. Here, the mean values for each variable obtained from the biotypes belonging to the same group are reported. Error bars indicate the standard deviation for each group and for each variable. L^* indicates lightness, a^* indicates red (+ a) to green (- a) colours, and b^* indicates yellow (+ b) to blue (- b) colours.

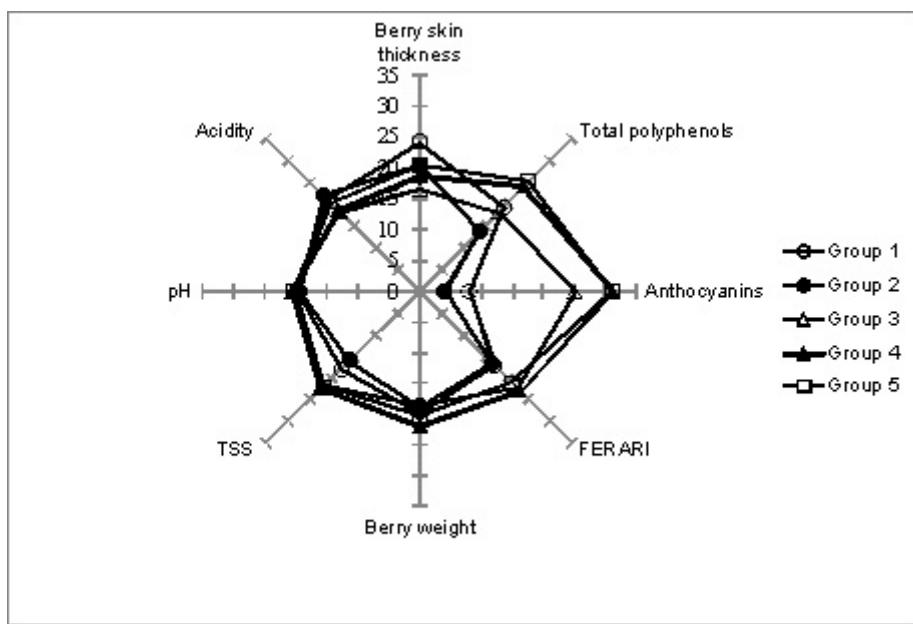


FIGURE 5

Diagram showing the profile of each Cannonau biotype group obtained by cluster analysis after normalisation of fluorescence, texture and chemical data. TSS means total soluble solids.

present in the literature on the use of CIELab parameters for discriminating between biotypes of the same variety.

PCA analysis, performed using the variables that accounted for the majority of variation between the biotypes (11 in total), revealed the relationships between the variables and the 85 biotypes and led to the identification of a group composed of eight biotypes characterised by very distinct traits compared with the remaining population (Fig. 2a). These biotypes are characterised by significant differences in berry colour and technological and phenolic maturity. In relation to colour, all of them showed high positive values for the a^* coordinate, indicating a colour between pink and red, and high positive value for the b^* coordinate, indicating a tendency towards yellow, typical of unripe grapes (Fig. 2b).

Moreover, the L^* values were also high for these biotypes, and the values for anthocyanin content were low. This result is supported by other reports in the literature (Liang *et al.*, 2011; Diago *et al.*, 2013) that indicate the correlation between high L^* values and low levels of anthocyanins; indeed, these biotypes exhibited low levels of total anthocyanins, as well as low FERARI values and low values of TSS, confirming their relative unripeness.

The relationship between the CIELab and fluorescence parameters of grapes at the time of commercial harvest was studied by Bramley *et al.* (2011) in order to characterise within-vineyard variations in grape berry anthocyanins, using an on-the-go sensing approach focused on anthocyanin content and using two indexes only: ANTH_RG and the

TABLE 3
Mean, standard deviation (SD) and coefficient of variation (% CV) of the variables for each group obtained by cluster analysis.

Groups	Berry skin thickness (mm)			Berry weight (g)			Berry a*			Total polyphenols			Total anthocyanins			TSS			pH			Acidity				
	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV	Mean	SD	% CV		
Group 1 (5 biotypes)	Mean	120.75 ^a	1.62 ^c	1.92 ^{bc}	0.09	0.09	1.76	0.25	0.57	10.82 ^a	1.09 ^b	0.09	707.02 ^b	30.79 ^a	1.09 ^b	39.70 ^c	17.64 ^b	1.09 ^b	4.24 ^b	4.55 ^b	1.57	0.05	0.67			
	Average SD	12.54	0.4	5.6	4.9	5.6	5.7	2.3	51.9	23.9	20.0	8.9	1.2	1.2	15.68 ^b	4.19 ^b	4.75 ^b	14.8								
Group 2 (3 biotypes)	Mean	102.29 ^{bc}	1.58 ^c	1.83 ^{bc}	0.16	0.26	2.73	0.51	3.07	34.47 ^b	8.68 ^b	6.06 ^a	506.97 ^b	20.02 ^c	20.02 ^c	112.23	20.37	4.26	0.03	0.19						
	Average SD	3.88	3.8	10.3	14.2	7.9	5.9	50.7	22.1	10.18	125.65 ^b	661.12 ^b	125.65 ^b	21.62 ^a	4.42 ^a	4.42 ^a	3.93 ^a									
Group 3 (34 biotypes)	Mean	83.06 ^a	2.14 ^a	1.80 ^c	0.16	0.17	1.52	1.29	0.48	28.28 ^c	4.07 ^c	-1.66 ^c	38.50	131.49	131.49	38.50	1.93	0.12	0.26							
	Average SD	10.26	0.26	0.15	0.17	0.17	1.52	1.29	0.48	9.3	5.4	31.8	28.9	19.9	19.9	30.6	8.9	2.7	6.6							
Group 4 (31 biotypes)	Mean	92.97 ^c	2.21 ^a	2.14 ^a	0.16	0.16	1.85	0.66	0.67	27.11 ^d	3.24 ^d	-1.92 ^c	882.47 ^a	155.99 ^a	155.99 ^a	22.35 ^a	4.42 ^a	4.42 ^a	3.99 ^a							
	Average SD	9.83	0.12	0.12	0.16	0.16	1.85	0.66	0.67	7.3	6.8	20.4	-34.7	134.91	134.91	36.17	1.25	0.08	0.36							
Group 5 (12 biotypes)	Mean	102.43 ^b	2.02 ^b	1.97 ^b	0.22	0.27	1.96	0.94	0.99	28.21 ^{cd}	3.89 ^{cd}	-1.47 ^c	923.33 ^a	155.03 ^a	155.03 ^a	21.69 ^a	4.36 ^a	4.36 ^a	4.40 ^a							
	Average SD	17.21	0.22	0.22	0.27	0.27	1.96	0.94	0.99	10.7	13.8	7.0	24.1	-67.6	168.05	168.05	50.14	1.36	0.08	0.51						

* Different letters within the same column mean significant differences according to LSD Fisher's test ($p < 0.001$)

TABLE 4

Pearson's correlation coefficients between the variables ($P < 0.05$) used to identify the differences among the 85 biotypes.

Variables	Berry skin thickness			Total polyphenols			anthocyanins			FERRARI			Berry weight			TSS			pH			Acidity			Production/ha		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	FERRARI	a*	b*	L*	a*	b*	FERRARI	a*	b*	TSS	a*	b*	L*	a*	b*	TSS	a*	b*
L*	1.00	0.77	0.99	0.44	-0.84	-0.93	-0.91	-0.91	-0.91	-0.58	-0.97	-0.93	0.85	0.85	0.89	-0.93	-0.93	0.85	0.85	0.85	0.78	0.78	0.57	0.78	0.78	0.57	
a*	0.77	1.00	0.72	0.81	-0.61	-0.94	-0.94	-0.94	-0.94	-0.40	-0.90	-0.90	0.78	0.78	0.78	-0.95	-0.95	0.78	0.78	0.78	0.83	0.83	0.92	0.83	0.83	0.92	
b*	0.99	0.72	1.00	0.39	-0.81	-0.89	-0.87	-0.87	-0.87	-0.48	-0.95	-0.95	0.83	0.83	0.83	-0.76	-0.76	0.60	-0.74	-0.74	0.76	0.76	0.76	0.45	0.45	0.45	
Berry skin thickness	0.44	0.81	0.39	1.00	-0.05	-0.59	-0.59	-0.59	-0.59	0.66	0.78	0.79	0.64	0.64	0.64	0.66	0.66	0.78	0.79	0.79	0.64	0.64	0.64	-0.45	-0.45	-0.53	
Total polyphenols	-0.84	-0.61	-0.81	-0.05	1.00	-0.05	1.00	1.00	1.00	0.83	0.66	0.66	0.78	0.78	0.78	0.76	0.76	0.78	0.79	0.79	0.64	0.64	0.64	0.76	0.76	0.45	
Total anthocyanins	-0.93	-0.94	-0.87	-0.76	0.66	0.95	1.00	1.00	1.00	0.83	1.00	1.00	0.95	0.55	0.98	0.98	0.98	0.55	0.93	-0.79	-0.79	-0.79	-0.71	-0.71	-0.71		
FERARI	-0.91	-0.94	-0.87	-0.76	0.66	0.95	1.00	1.00	1.00	0.83	1.00	1.00	0.95	0.47	0.97	0.97	0.97	0.47	0.99	-0.93	-0.79	-0.79	-0.79	-0.79	-0.79	-0.79	
Berry weight	-0.58	-0.40	-0.48	0.06	0.78	0.55	0.47	0.47	0.47	0.83	0.51	0.51	0.39	0.39	0.39	0.32	0.32	0.51	0.51	0.51	0.39	0.39	0.39	-0.25	-0.25	-0.25	
TSS	-0.97	-0.90	-0.95	-0.60	0.79	0.98	0.51	0.51	0.51	0.79	0.97	0.51	1.00	0.97	0.97	0.97	0.97	0.51	1.00	0.97	-0.87	-0.87	-0.87	-0.83	-0.83	-0.83	
pH	-0.93	-0.90	-0.91	-0.74	0.64	0.93	0.39	0.39	0.39	0.93	0.99	0.39	0.97	1.00	0.99	0.99	0.99	1.00	0.99	-0.95	-0.86	-0.86	-0.86	-0.86	-0.86	-0.86	
Acidity	0.85	0.78	0.83	0.76	-0.45	-0.79	-0.93	-0.93	-0.93	-0.32	-0.87	-0.87	-0.95	1.00	1.00	0.90	0.90	1.00	1.00	0.90	1.00	1.00	1.00	1.00	1.00	1.00	
Production/ha	0.89	0.57	0.92	0.45	-0.53	-0.71	-0.79	-0.79	-0.79	-0.25	-0.83	-0.83	-0.86	-0.86	-0.86	-0.86	-0.86	-0.79	-0.79	-0.79	1.00	1.00	1.00	1.00	1.00	1.00	

* Correlation coefficients that are significant for $P < 0.05$ are reported in bold. All the variables responsible for the differences between the Cannonau biotypes were used. As yield is a very important factor that influences grape quality, production per hectare was added to reveal possible correlations with the parameters analysed.

FERARI index. When the value of the ANTH_RG index is high, the anthocyanin content is low (Cerovic *et al.*, 2008; Ghozlen *et al.* 2010a; 2010b). ANTH_RG, when used alone, was not able to produce a clear discrimination within the Cannonau population, whereas the FERARI index significantly contributed towards separating the eight biotypes in the PCA, as reported above (Figs 2a and 2b). Moreover, when applying cluster analysis to the results of these methodologies, five distinct groups were identified (Fig. 3), characterised by different values of CIELab parameters, FERARI index and anthocyanins (Table 3 and Figs 4 and 5). FERARI index values showed a significant and highly positive correlation with total anthocyanins, TSS and pH, and a significant negative correlation with L*, a* (Table 4) and total acidity. The FERARI index thus represents a very good index for assaying grape anthocyanins, confirming the results of Bramley *et al.* (2011), and for providing a gauge of grape colour.

Instrumental texture analysis of grapes has been in use since the 1980s to describe the changes in the mechanical properties of grape berries during ripening (Rolle *et al.*, 2012). As is well known, berry composition at the time of harvesting is a determinant of the resulting wine quality. However, the use of instrumental texture analysis for the rapid prediction of phenolic maturity in grapes is more recent (Río Segade *et al.*, 2008, Rolle *et al.*, 2011); indeed, until now, no studies have addressed the potential differences in texture between biotypes belonging to the same variety, while the effectiveness of texture parameters as varietal markers has been demonstrated (Rolle *et al.*, 2012). Although the mechanical characteristics of grapes may change from year to year due to seasonal variations (Letaief *et al.*, 2008), berry skin thickness and the mechanical properties of seeds were evaluated for this paper at the moment of commercial harvesting to investigate the presence of possible differences between the 85 biotypes.

The mechanical properties of seeds were not able to differentiate between the biotypes, thus attention was focussed on berry skin thickness. Data obtained from the berry skin thickness analysis of the 85 Cannonau biotypes were lower than some data reported in the literature (Río Segade *et al.*, 2008; Giacosa *et al.*, 2013) for other varieties. In fact, cv. Cannonau is well known for its thin skin, so this variety, according to OIV code number 228 (berry: thickness of skin) of the OIV Descriptor List (2001), could be ranged between note 1 (very thin) and note 3 (thin). Many studies have investigated the correlation between berry skin thickness and anthocyanin extractability in relation to various cultivars, and the general consensus is that thinner skins produce a greater release of anthocyanins (Río Segade *et al.* 2008; 2011a). In this paper, only the biotypes included in the first group, characterised by low values of anthocyanins, FERARI and TSS and high values of L*, a* and b* coordinates, revealed high values for skin thickness, even though the same values were also reported for group 5, characterised by high values of TSS, anthocyanins and FERARI, suggesting that the parameter berry skin thickness is not able to differentiate between the Cannonau biotypes; hence, it was not considered a good variable for differentiating between cultivars, as also

concluded by Río Segade *et al.* (2008). However, this result does not correspond with the data of Rolle *et al.* (2012), who found significant differences between the different Italian cultivars studied, and attributed the variations observed in the same cultivar to possible clonal differences. Finally, no correlations were present between berry skin thickness and the CIELab parameters, technological maturity parameters or FERARI values, suggesting that this variable, when used alone, is not useful for predicting the phenolic and/or technological maturity of grapes (Río Segade *et al.* 2011b).

Concerning berry weight, several papers have reported a relationship between this parameter and the other components responsible for the quality of the must, whereas others have reported that further factors, such as climatic conditions and cultivation techniques, are likely to play more significant roles in determining grape composition (Ferrer *et al.*, 2014). It is well known that grape variety and vintage are responsible for the differences in berry weight, and that TSS content seems to be dependent on berry weight, although a correlation was found if sugars were expressed as g/berry. In the present study we did not find any correlation between TSS, expressed in °Brix, and berry weight. Thus, the present data confirm previous findings that show that total anthocyanin content is independent of berry weight (Ferrer *et al.*, 2014). In fact, no significant correlation was found between total anthocyanins and berry weight.

In addition to berry size, grape yield is widely acknowledged as a determining factor of wine grape quality. It is generally considered that must quality decreases as the yield increases. In our study, production per hectare was positively correlated with the L* and b* parameters and with titratable acidity, showing that, when the production is high, the 'lightness' of the berry colour is high, which tends towards yellow, resulting in less mature grapes. This data was confirmed by grape acidity, which was significantly and positively correlated with production per hectare.

CONCLUSIONS

In our findings we observed extensive agronomical variability within the biotypes of the same Cannonau variety. Specifically, significant differences in titratable acidity and total soluble solids (TSS) were identified between the 85 biotypes, as well as different anthocyanin and polyphenol contents. Until now, fluorescence, texture and colorimetry techniques have mainly been used as a means to evaluate the best data for grape harvest, and only recently have these methodologies been used to differentiate between table and wine grape varieties. Here, for the first time, these techniques were used to evaluate variability within the Cannonau variety, in comparison with data obtained using the standard chemical analyses commonly used to evaluate the stage of grape technological and phenolic maturity. In particular, the application of fluorescence and colorimetric techniques was able to separate the biotypes into distinct groups, thereby providing a rapid and non-invasive tool for the assessment of wine grape quality. The proposed techniques could also help winemakers identify the most appropriate harvesting period for each biotype.

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