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Seed morpho-colorimetric analysis by computer vision: a helpful tool to identify grapevine (*Vitis vinifera* L.) cultivars

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Abstract

Background and Aims: The Sardinian wine heritage consists of 151 cultivars; however, because of the different dialects within the same region, many of these cultivars can be considered as false attributions (synonyms/homonyms). The aim of this study was to use seed morpho-colorimetric features to discriminate among the grapevine cultivars and identify synonymy groups.

Methods and Results: Over two seasons, 230 grapevine accessions belonging to 115 Sardinian native cultivars (54 black berries and 61 white berries) were collected. Seed images were acquired and analysed. Thirty-three morpho-colorimetric attributes and 80 elliptic Fourier descriptors were assessed. The data were analysed by applying linear discriminant analysis to implement statistical classifiers able to identify the landraces. Fourteen black berry synonymy groups and eight independent cultivars, and 15 white berry synonymy groups and nine independent cultivars, were identified.

Conclusions: These results confirmed the synonymy groups previously proposed and improved some of them with other local cultivars.

Significance of the Study: Despite different historical and cultural consumption purposes, some cultivars belong to the same synonymy groups, causing apparent incongruence. Nevertheless, the results are consistent with the geographical distribution of the cultivars and with the historical and cultural knowledge in Sardinia.

Keywords: *biometric characterisation, EFDs, image analysis, Sardinia, seeds (pips), synonymy group*

Introduction

Sardinia, with an area of 24 089 km^2, is the second largest island in the Mediterranean Sea after Sicily, and because of its geographical position and extremely diversified ecological conditions hosts an ideal environment for the growth of wild grape [*Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel) Hegi] and grapevine species (*V. vinifera* L. subsp. *vinifera*) (Zecca et al. 2010).

During the quaternary glaciations, the island was a refuge for *V. sylvestris*, but this species did not play any role in the recolonisation of wild grapes in Central Europe because of its distance from the European continent (Grassi et al. 2008). Insularity and the presence of abundant populations of wild grape allowed independent domestication (Snoussi et al. 2004, Imazio et al. 2006). Grassi et al. (2003) highlighted how the gene pool of two autochthonous cultivars (Bovale Muristellu and Bovale Murru) is similar to that of wild grapes collected in the nearby countryside area of Nuoro, providing evidence that Sardinia is a plausible secondary centre of domestication of *V. sylvestris*. Phenomena of independent domestication of different autochthonous grapevine cultivars have been found in other Mediterranean regions, such as Spain and Greece (Arroyo-García et al. 2002), where more than 70% of autochthonous cultivars appear to descend from western wild grape populations (Arroyo-García et al. 2006).

The large number of traditional cultivars, considered as local cultivars, characterised by peculiar morphological and chemical characteristics (Castia et al. 1992, Calò et al. 2002), is the result of different events, including direct domestication of wild grape, crosses between local cultivars, and the importation of agricultural techniques and cultivars from different ethnic groups who colonised the Island (De Mattia et al. 2007). In a recent study, De Mattia et al. (2009) showed that no genetic relationships exist between the cultivars Vernaccia (or Granaccia) of the Sardinian group, and Garnacha Tinta and Garnacha Blanca of the Spanish group, and neither of them originated from wild grape in Sardinia, but rather through selection processes. Moreover, in the same study, on the basis of molecular analysis, Cannonau of Sardinia and the Spanish Garnacha Tinta are considered synonymous (De Mattia et al. 2009).

Currently, grapevine 'cultivars' in Sardinia amount to 151 (Lovicu et al. 2010), they are often circumscribed in a small area (Nieddu 2011) and are distributed homogeneously throughout the region (Lovicu 2007). Numerous cultivars can be considered as false attributions (De Mattia et al. 2007, Orrù et al. 2012) because of the existence of different dialects within the same territory (Lovicu et al. 2010).

Grapevine seeds are highly polymorphic and are important taxonomic descriptors within the genus *Vitis* L. (Rivera et al. 2007), and have a role in the distribution and domestication processes of the wild grapevine in many archaeological discoveries (Zohary and Spiegel-Roy 1975, Zohary and Hopf 1993, 2000, Olmo 1995, Zohary 1996, McGovern 2003, This et al. 2006, Forni 2007), and in the study of the identification and

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grouping of diasporas of *Vitis* (Rivera et al. 2007, Gong et al. 2010, Terral et al. 2010, Orrù et al. 2012, 2013).

Recent studies have focused on the identification and grouping of diasporas of *Vitis* on the basis of biometric features. Using an electronic caliper, Rivera et al. (2007) measured 11 morphometric variables on 142 different types of grape: five taxa of *Vitis*, 92 cultivars of *V. vinifera*, 12 feral/wild populations and four hybrid rootstock cultivars. The data obtained were subjected to cluster analysis, placing feral/wild populations and related cultivars in their respective clusters, but missing a cluster of wild European grapevine. Applying the elliptic Fourier descriptors (EFDs) method, Terral et al. (2010) compared well-preserved archaeological seeds, found in southern France and dated back to the I BC, with the same European modern cultivars and wild individuals. Also, Gong et al. (2010) used digital images to analyse the morphometry of some fossil seeds of *Vitis*, dug up from the Gray Fossil Site (N-E Tennessee, USA) and dated to the latest Miocene–earliest Pliocene, placing them in three morphotaxa on the basis of 11 measured characters. Orrù et al. (2013), through linear discriminant analysis (LDA) of the biometric parameters among the three groups (archaeological, *V. vinifera* and *V. sylvestris* seeds), showed the greatest similarity of the archaeological seeds of *V. vinifera*, in particular, to the white berry cultivars rather than to the black berry cultivars. According to the authors, these findings suggest that *V. vinifera* was probably already used to produce wine and/or to preserve foodstuffs as grape, also supporting the traditional production of white grapes in particular areas of Sardinia.

Considering the convincing results achieved with the synonymy study of grapevine cultivars conducted by Orrù et al. (2012) on the basis of 113 morpho-colorimetric features compared with the previous molecular simple sequence repeat analysis conducted by De Mattia et al. (2007) on the same material, the aim of this study was to use the same seed morpho-colorimetric features and EFDs obtained by image analysis to implement dedicated statistical classifiers able to discriminate among the studied cultivars of the grapevine, also considering specific aspects as grape colour (black or white) and end use (tablegrape or winegrape, and table wine, moscato wine or dessert wine).

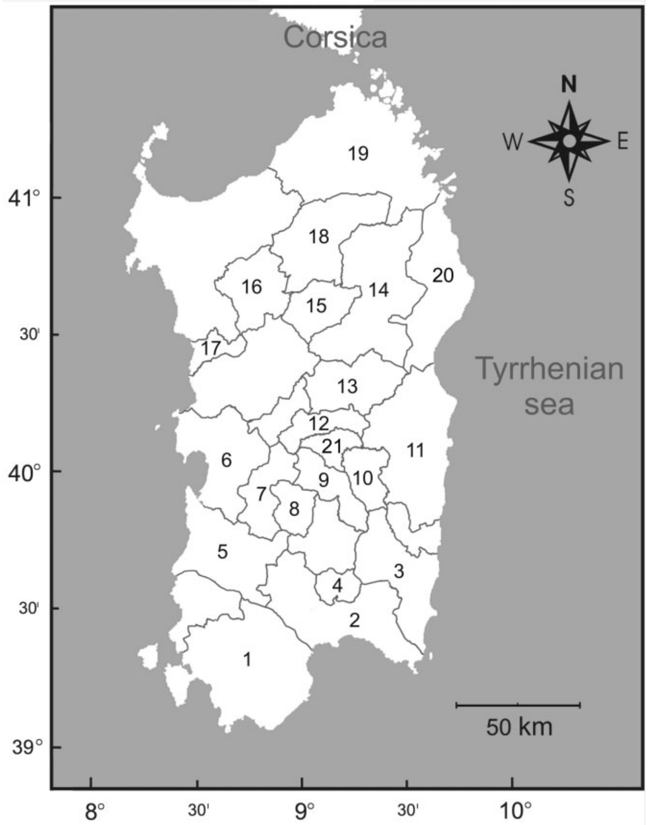

Figure 1. Historical distribution regions in Sardinia of the *Vitis vinifera* L. cultivars studied: 1, Sulcis; 2, Campidano di Cagliari; 3, Sarrabus e Gerrei; 4, Parteòlla; 5, Campidano di Sanluri; 6, Campidano di Oristano; 7, Barigadu; 8, Marmilla; 9, Sarcidano; 10, Barbagia di Seùlo; 11, Ogliastra; 12, Mandrolisai; 13, Barbagia di Ollolai; 14, Barbagia di Nuoro; 15, Goceano; 16, Meilogu; 17, Planargia; 18, Monteacuto; 19, Gallura; 20, Baronie; 21, Barbagia di Belvì.

Materials and methods

Seed material

Seeds of 115 native cultivars of *V. vinifera* representing a major part of the agrobiodiversity of the Sardinia Island were collected as part of the AGRIS germplasm collections (Agenzia per la Ricerca in Agricoltura della Regione Sardegna) of Ussana (Sardinia, Italy) during the harvest years 2008/09 and 2009/10, for a total of 230 seed accessions (Figure 1). All cultivars studied are listed in Table 1, reporting the grape type (TG for tablegrape, WG for winegrape and TG/WG for dual-purpose grape), the wine type (TW for table wine and DW for moscato or dessert wine), the berry colour (B for black and W for white), a code arbitrarily chosen, the cultivar name and the relative distribution in the Sardinian region. Grapes were sampled at the time of maximum concentration of sugar in the pulp (ripeness) corresponding to the complete morphological development (Failla 2007). In order to study samples sufficiently representative of the morpho-colorimetric variability (Guarino et al. 1995, Bacchetta et al. 2008b) for each cultivar, 10 bunches were collected from ten individual vines. Subsequently, 1030 berries, depending on bunch and berry morphology, were selected from the central part of each bunch. According to the Organisation Internationale de la Vigne et du Vin descriptor list for grape cultivars and *Vitis* species (Organisation Internationale de la Vigne et du Vin 2009), undeveloped seeds (stenospermocarpic) were rejected and not used for the analysis.

Seed size, shape and colour analysis

Digital images of seed samples were acquired with a flatbed scanner (Epson GT-15000, Epson America, Long Beach, CA, USA) with a digital resolution of 400 dpi and a scanning area not exceeding 2048 × 2048 pixels. Images were acquired before drying the seeds at 15°C to 15% of RH to avoid spurious variation in dimension, shape and colour. Moreover, before image acquisition, the scanner was calibrated for colour matching following the protocol of Shahin and Symons (2003), as suggested by Venora et al. (2009b). Samples consisting of 100 seeds were captured and used for the digital image analysis. In order to represent the whole variability of each accession, the image of the seed samples was acquired three times, randomly disposing the seed samples on the flatbed tray between each image. Over 87 000 statistical cases (seeds) were analysed in total.

Digital images of seeds were processed and analysed with the software package KS-400 V. 3.0 (Carl Zeiss Vision, Oberkochen, Germany). A macro specifically developed for the characterisation of seeds (Bacchetta et al. 2008a), and later modified to measure a further 20 morpho-colorimetric seed features (Mattana et al. 2008), was adapted to automate all the analysis procedures, reducing the execution time and mistakes

Table 1. Characteristics of *Vitis vinifera* L. Sardinian cultivars in the study, including information about consumption end use, berry colour, cultivar name and regional distribution.

Colour	Code	Cultivar	Distribution in Sardinia
Tablegrape			
B	ApN	Apesorgia nera	Campidano di Cagliari
	Axt	Axina de tres bias	Campidano di Cagliari
	AcA	Aghina'e cressia di Abbasanta	Barigadu
	ObO	Ocre e boe di Orosei	Baronie
W	Cob	Corniola Bianca	Parteòlla
	GlE	Galoppu di Escalaplano	Sarrabus-Gerrei
	GlN	Galoppu di Nurri	Sarcidano
	TtG	Tittiacca di Gonnos	Campidano di Sanluri
	TtS	Tittiacca verde di Serramanna	Campidano di Cagliari
	AGA	Aghina de Gerusalemme di Abbasanta	Barigadu
	CfO	Corofulu di Oliena	Barbagia di Ollolai
	OlT	Olopo di Triei	Ogliastra
	TtM	Tittibacchina di Mamoiada	Barbagia di Ollolai
Tablegrape/winegrape			
B	PrN	Primidivu Nieddu	Meilogu
W	PsN	Pascale di Nurri	Sarcidano
	BnC	Bianca di Chilivri	Baronie
Winegrape			
B	BrS	Barbera Sarda	Parteòlla
	CgN	Cagnulari di Nurri	Sarcidano
	Cn	Cannonau	Barbagie
	Cs	Carenisca	Sulcis
	CcM	Caricagiola di Monti	Monteacuto
	FlG	Falso Gregu	Campidano di Cagliari
	GNC	Gregu Nieddu del Campidano	Campidano di Cagliari e Oristano
	MN	Monica nera	Campidano di Cagliari
	Mu	Muristellu	Mandrolisai
	NeB	Nera di Bosa	Planargia
	Vrt	Vertudi	Sulcis
	AnL	Aniga di Lanusei	Mandrolisai
	CnS	Cannonau nero di Sestu	Campidano di Cagliari
	CrT	Canulare di Triei	Ogliastra
	FlC	Falso Canulare di Triei	Ogliastra
	FdS	Fiudedda di Sini	Marmilla
	Grm	Girò morbido di Serri	Sarcidano
	GNS	Gregu Nieddu di Serramanna	Campidano di Cagliari
	LxN	Licronaxiu nero Nuraxinieddu	Campidano di Oristano
	ME	Monica di Escalaplano	Sarrabus e Gerrei
	MSl	Monica di Seulo	Barbagia di Seùlo
	MSr	Monica di Sorgono	Mandrolisai
	MuS	Muristeddu di Sorgono	Mandrolisai
	NsA	Nasco nero di Abbasanta	Barigadu
	NeA	Nera di Abbasanta	Barigadu
	NeE	Nera di Escalaplano	Sarrabus-Gerrei
	NeS	Nera di Sini	Marmilla
	NeM	Nera glabra di Modolo	Planargia
	NeL	Nera liscia di Montresta	Planargia
	NeT	Nera tomentosa	Planargia
	NSl	Niedda carta di Seulo	Barbagia di Seùlo
	NPS	Nieddu Pedra Serra	Campidano di Sanluri
	NNr	Nieddu mannu di Nurri	Sarcidano
	NPt	Nieddu mannu di Pattada	Monteacuto
	NPl	Nieddu Polchino	Monteacuto
	NrS	Nuragus nero di Sini	Marmilla
	SpS	Salude e passa di Serramanna	Campidano di Cagliari
	TrS	Teresina di Sini	Marmilla
	CnB	Cannonatu anticu di Bitti	Barbagia di Nuoro
	Ms	Mustiosa	Barbagia di Nuoro
	NeO	Nera di Orosei	Baronie
	NeJ	Nera di Janna Ritha	Baronie

Table 1. (continued)

Colour	Code	Cultivar	Distribution in Sardinia
W	AxF	Axina de Francia	Campidano di Cagliari
	BnM	Bianca pelosa di Montresta	Planargia
	CnT	Cannonau bianco di Trieri	Ogliastra
	Cl	Culupuntu	Ogliastra
	Lx	Licronaxiu	Campidano di Oristano
	Mz	Mizu	Campidano di Cagliari, Oristano e Sanluri, Marmilla
	Nr	Nuragus	Campidano di Cagliari
	MB	Monica Bianca	Campidano di Cagliari, Oristano e Sanluri
	Sm	Semidano	Marmilla
	Sn	Sinnidanu	Baronie
	AbT	Albacanna di Triei	Ogliastra
	Al	Alicante	Sardegna
	BnL	Bianca di Lodine	Barbagia di Ollolai
	BnP	Bianca di Padria	Meilogu
	CbS	Caddiu bianco di Serri	Sarcidano
	ClS	Calabresa di Seulo	Barbagia di Seùlo
	CuU	Cuccuau di Ula	Barigadu
	LcA	Lacconarzu di Abbasanta	Barigadu
	LgA	Luglienca di Abbasanta	Barigadu
	NrA	Nuragus Arrubiu	Campidano di Cagliari
	Nrt	Nuragus Moscatello	Sarcidano
	Nrd	Nuragus Muscadeddu	Sarrabus-Gerrei
	NrR	Nuragus rosso rompizzolla	Campidano di Cagliari
	PzU	Panzale di Ula	Barigadu
	RtM	Retagliaddu di Monti	Monteacuto
	CnO	Cannonau bianco di Oliena	Barbagie
Moscato/dessert wine			
B	Gr	Girò	Campidano di Cagliari
	GrG	Girò di Gonnos	Campidano di Sanluri
	Grs	Girò scuro di Serri	Sarcidano
	MnG	Moscatello nero di Genuri	Marmilla
	MrS	Moscatello rosso di Seulo	Barbagia di Seùlo
	MnS	Moscato nero di Sini	Marmilla
	MnU	Moscato nero di Ulatirso	Barigadu
W	ArB	Aregu biancu	Barbagia di Seùlo
	Av	Arvesiniadu	Goceano
	Cd	Codronisca	Campidano di Cagliari
	GzM	Granatza di Mamoiada	Barbagia di Ollolai
	GzS	Granatza Aregu di Seulo	Barbagia di Seùlo
	GzG	Granazza di Garaumele	Barbagia di Ollolai
	GB	Gregu bianco	Campidano di Cagliari e Oristano
	Mb	Moscatello bianco	Campidano di Cagliari, Oristano e Sanluri
	MbG	Moscato bianco di Genuri	Marmilla
	MFn	Moscato di Fonni	Barbagia di Ollolai
	MPt	Moscato di Pattada	Monteacuto
	Ns	Nasco	Campidano di Cagliari
	RmS	Remungiau di Serri	Sarcidano
	VrR	Vernaccia di S. Rosalia	Ogliastra
	VrS	Vernaccia di Solarussa	Campidano di Oristano
	AvB	Alvaranzeniadu bianco di Bosa	Planargia
	MbS	Moscato bianco di Sini	Marmilla
	MLd	Moscato di Lodine	Barbagia di Ollolai
	MT1	Moscato di Tempio 1	Gallura
	MT2	Moscato di Tempio 2	Gallura
	MSP	Moscato su pinu di Mamoiada	Barbagia di Ollolai
	VrV	Vernaccia bidri di Villasor	Campidano di Cagliari
	VrE	Vernaccia di Escalaplano	Sarrabus-Gerrei
	VrO	Vernazza di Orosei	Baronie

B, black; W, white.

in the analysis process (Grillo et al. 2010). This macro was further enhanced by adding algorithms able to compute the EFDs for each seed analysed, obtaining a further 80 parameters of value to discriminate among the studied cultivars of *Vitis* (Table 2) (Bacchetta et al. 2009, 2010, Terral et al. 2010, Orrù et al. 2013). For each seed, 113 morpho-colorimetric features were measured.

Statistical analysis

Data assembled from the morpho-colorimetric and EFDs were analysed with the SPSS software package release 15.0 (IBM Corporation, Armonk, NY, USA). Stepwise LDA was used to compare the investigated cultivar groups, a well-known method for dimensionality reduction and classification that projects high-dimensional data onto a low-dimensional space where the data achieve maximum class separability (Fukunaga 1990, Duda et al. 2000, Hastie et al. 2001). The LDA approach is commonly used to classify/identify unknown groups characterised by quantitative and qualitative variables (Venora et al. 2009a, Bacchetta et al. 2011a,b, Grillo et al. 2011, 2012). This is possible due to the derived discriminant functions that are linear combinations of the original morpho-colorimetric features, where the coefficients are from the transformation matrix. The optimal projection or transformation in classical LDA is obtained by finding the combination of predictor variables with the aim of minimising the within-class distance and maximising the between-class distance simultaneously, thus achieving maximum class discrimination (Hastie et al. 2001, Holden et al. 2011, Rencher and Christensen 2012, Kuhn and Johnson 2013). The original LDA formulation, also known as the Fisher linear discriminant analysis (Fisher 1936, 1940), deals with binary classifications. The key idea in LDA is to look for a direction that separates the class means well (when projected onto that direction) while achieving a small variance around these means (Jieping 2007). The selection of the original features is carried out by a stepwise procedure. The stepwise method identifies and selects the most statistically significant features among them to use for the seed sample identification,

Table 2. List of morpho-colorimetric attributes measured on seeds, excluding the 80 elliptic Fourier descriptors calculated according to Hâruta (2011).

Attribute		Description
Area	A	Seed area (mm^2)
Perimeter	P	Seed perimeter (mm)
Convex perimeter	P_{conv}	Convex perimeter of the seed (mm)
Crofton's perimeter	P_{Crof}	Perimeter of the seed calculated using the Crofton's formula (mm)
Perimeter ratio	P_{conv}/P_{Crof}	Ratio between convex and Crofton's perimeters
Max diameter	D_{max}	Maximum diameter of the seed (mm)
Min diameter	D_{min}	Minimum diameter of the seed (mm)
Feret ratio	D_{min}/D_{max}	Ratio between minimum and maximum diameters
Shape factor	Sf	Seed shape descriptor = $(4 \times \pi \times \text{area})/\text{perimeter}^2$ (normalised value)
Roundness factor	Rf	Seed roundness descriptor = $(4 \times \text{area})/(\pi \times \text{max diameter}^2)$ (normalised value)
Equivalent circular diameter	Ecd	Diameter of a circle with an area equivalent to that of the seed (mm)
Maximum ellipse axis	EA_{max}	Maximum axis of an ellipse with equivalent area (mm)
Minimum ellipse axis	EA_{min}	Minimum axis of an ellipse with equivalent area (mm)
Mean red channel	R_{mean}	Red channel mean value of seed pixels (grey level)
Red SD	R_{sd}	Red channel standard deviation of seed pixels
Mean green channel	G_{mean}	Green channel mean value of seed pixels (grey level)
Green SD	G_{sd}	Green channel standard deviation of seed pixels
Mean blue channel	B_{mean}	Blue channel mean value of seed pixels (grey level)
Blue SD	B_{sd}	Blue channel standard deviation of seed pixels
Mean hue channel	H_{mean}	Hue channel mean value of seed pixels (grey level)
Hue SD	H_{sd}	Hue channel standard deviation of seed pixels
Mean lightness channel	L_{mean}	Lightness channel mean value of seed pixels (grey level)
Lightness SD	L_{sd}	Lightness channel standard deviation of seed pixels
Mean saturation channel	S_{mean}	Saturation channel mean value of seed pixels (grey level)
Saturation SD	S_{sd}	Saturation channel standard deviation of seed pixels
Mean density	D_{mean}	Density channel mean value of seed pixels (grey level)
Density SD	D_{sd}	Density channel standard deviation of seed pixels
Skewness	S	Asymmetry degree of intensity values distribution (grey level)
Kurtosis	K	Peakness degree of intensity values distribution (densitometric units)
Energy	H	Measure of the increasing intensity power (densitometric units)
Entropy	E	Dispersion power (bit)
Density sum	D_{sum}	Sum of density values of the seed pixels (grey level)
Square density sum	SqD_{sum}	Sum of the squares of density values (grey level)

using three statistical variables: tolerance, *F*-to-enter and *F*-to-remove. The tolerance value indicates the proportion of a variable variance not accounted for by other independent variables in the equation. Values of *F*-to-enter and *F*-to-remove define the power of each variable in the model, and they are helpful to describe what happens if a variable is inserted and removed, respectively, from the current model. This selective process starts with a model that does not include any of the original morpho-colorimetric features. At each step, the feature with the largest *F*-to-enter value that exceeds the entry criteria chosen ($F \geq 3.84$) is added to the model. The original features left out of the analysis at the last step have *F*-to-enter values smaller than 3.84, so no more are added. The process is automatically stopped when no remaining morpho-colorimetric features increase the discrimination ability (Venora et al. 2007, Grillo et al. 2012).

Finally, a cross-validation procedure was applied to verify the performance of the identification system, testing individual unknown cases and classifying them on the basis of all others. This procedure, also called rotation estimation (Picard and Cook 1984, Kohavi 1995), was applied to evaluate the performance and to validate any classifier. The validation procedure used here is the leave-one-out cross-validation. It involves using a single case from the original sample set as the validation dataset and the remaining cases as the training set. Each case is classified into a group according to the classification functions computed from all the data except the case being classified. The proportion of misclassified cases after removing the effect of each case one at a time is the leave-one-out estimate of misclassification (SPSS 2006).

Grouping procedure

Because of the large number of data, the discriminatory steps were executed distinguishing between black and white berry cultivars, allowing an easier and faster identification of the hypothetical synonymy groups. Moreover, preliminary comparisons were executed distinguishing for end use of the grapevine cultivars. Following historical records, it was possible to differentiate between tablegrape, winegrape and dual-purpose grape cultivars; these latter cultivars are normally considered as cultivars commonly used for wine production and/or as tablegrapes, and among table wine, moscato wine or dessert wine. In a final step, the preliminary hypothetical groups were compared all together, only maintaining a distinction between white and black berry cultivars.

Results and discussion

Using the same approach that has been applied many times to solve cases of homonymy and synonymy of grapevine cultivars on the basis of molecular markers (Lopes et al. 1999, 2006, Maletić et al. 1999, Sefc et al. 2000, Crespan and Milani 2001, Fossati et al. 2001, Crespan et al. 2006, Ergül et al. 2006, Moreno-Sanz et al. 2008, Santana et al. 2008, Boz et al. 2011), morpho-colorimetric features were used to identify hypothetical synonymy groups of *V. vinifera* cultivars.

Using historical and cultural information, the first statistical comparison was executed on the basis of consumption purpose, distinguishing among tablegrape, winegrape and dual-purpose cultivars. Table 3 shows the proportion of correct identification of the three grape categories, distinguishing for berry colour. The high overall proportion of correct identification of winegrape cultivars, both for black (96.5%) and for white (97.6%) berry cultivars, is because of the strong classification of winegrape seeds, which is the largest group of cultivars. In contrast, the low recognition performance for the tablegrape (35.3% for black and 40.0% for white berry cultivars) and dual-purpose (35.1% for black and 4.9% for white berry cultivars) classes probably ensues from the non-univocal popular origin of the information on consumption purpose, collected in different areas of the Sardinian region. This comparison is helpful in correcting the assemblage of the hypothetical synonymy groups.

Exclusive comparison of the tablegrape cultivars, distinguished by means of berry colour (Table 4), suggests that the four black berry cultivars – Apesorgia nera (ApN), Axina de tres bias (Axt), Aghina'e cressia di Abbasanta (AcA) and Ocre e boe di Orosei (ObO) – are independent of one another; it appears, however, that the white berry cultivars form into two small hypothetical groups. Tittiacca di Gonnos (TtG) and Tittiacca verde di Serramanna (TtS) show a high level of correct identification (75.6 and 75.7%, respectively), but both the cultivars erroneously attribute more than 21% with the other one. Similarly, Corofulu di Oliena (CfO), Galoppu di Escalaplano (GlE), Galoppu di Nurri (GlN) and Tittibacchina di Mamoiada (TtM) reveal a high proportion of misidentification, between 2.7 and 16.0%, interchanging seeds with one another (Table 4).

Table 3. Proportion of correct classification among the grapevine cultivars on the basis of consumption and distinguished by berry colour.

		Proportion of correct classification (%)			
		WG	TG	TG/WG	Total
Black berry cultivars	WG	**96.5 (33 725)**†	2.6 (904)	0.9 (315)	100.0 (34 944)
	TG	64.5 (2110)	**35.3 (1154)**	0.2 (8)	100.0 (3272)
	TG/WG	64.8 (386)	0.2 (1)	**35.1 (209)**	100.0 (596)
	Overall				**90.4 (38 812)**
White berry cultivars	WG	**97.6 (39 407)**	2.3 (944)	0.1 (38)	100.0 (40 389)
	TG	60.0 (3497)	**40.0 (2329)**	–	100.0 (5826)
	TG/WG	95.1 (855)	–	**4.9 (44)**	100.0 (899)
	Overall				**88.7 (47 114)**

The correct classification performance is highlighted in bold. †The number of seeds analysed (shown in parenthesis). TG, tablegrape; TG/WG, dual-purpose grape; WG, winegrape.

Table 4. Proportion of correct classification among the tablegrape cultivars, distinguished by berry colour.

Cultivar	Proportion of correct classification (%)				
Black berry cultivars					
	ApN	**Axt**	**AcA**	**ObO**	**Total**
ApN	**95.5 (1138)**†	4.0 (48)	0.5 (6)	–	100.0 (1192)
Axt	3.1 (37)	**92.1 (1095)**	3.8 (45)	1.0 (12)	100.0 (1189)
AcA	2.2 (13)	7.1 (42)	**90.7 (536)**	–	100.0 (591)
ObO	–	3.0 (9)	–	**97.0 (291)**	100.0 (300)
Overall					**93.5 (3272)**

White berry cultivars										
	Cob	**GlE**	**GlN**	**TtG**	**TtS**	**AGA**	**CfO**	**OlT**	**TtM**	**Total**
Cob	**90.5 (948)**	0.7 (7)	0.8 (8)	–	–	2.1 (22)	0.3 (3)	4.7 (49)	1.0 (11)	100.0 (1048)
GlE	0.3 (2)	**70.5 (422)**	16.0 (96)	–	–	1.5 (9)	2.7 (16)	0.2 (1)	8.8 (53)	100.0 (599)
GlN	–	10.7 (67)	**72.4 (452)**	–	–	0.6 (4)	5.8 (36)	–	10.4 (65)	100.0 (624)
TtG	1.8 (10)	–	–	**75.6 (428)**	21.7 (123)	0.4 (2)	–	0.5 (3)	–	100.0 (566)
TtS	0.8 (5)	0.2 (1)	–	21.1 (125)	**75.7 (449)**	1.5 (9)	–	0.5 (3)	0.2 (1)	100.0 (593)
AGA	0.7 (4)	1.5 (9)	1.0 (6)	–	–	**86.0 (516)**	2.0 (12)	7.5 (45)	1.3 (8)	100.0 (600)
CfO	–	3.8 (23)	9.2 (55)	–	–	0.7 (4)	**76.1 (456)**	1.5 (9)	8.7 (52)	100.0 (599)
OlT	8.5 (51)	0.5 (3)	1.5 (9)	–	–	7.0 (42)	2.2 (13)	**79.9 (477)**	0.3 (2)	100.0 (597)
TtM	–	12.2 (73)	9.0 (54)	–	–	0.7 (4)	8.8 (53)	0.7 (4)	**68.7 (412)**	100.0 (600)
Overall										**78.3 (5826)**

The correct classification performance is highlighted in bold. †The number of seeds analysed (shown in parenthesis). AcA, Aghina'e cressia di Abbasanta; AGA, Aghina de Gerusalemme di Abbasanta; ApN, Apesorgia nera; Axt, Axina de tres bias; Cob, Corniola Bianca; CfO, Corofulu di Oliena; GIE Galoppu di Escalaplano; GIN, Galoppu di Nurri; ObO, Ocre e boe di Orosei; OIT, Olopo di Triei; TtG, Tittiacca di Gonnos; TtM, Tittibacchina di Mamoiada; TtS, Tittiacca verde di Serramanna.

Table 5. Proportion of correct classification among the dual-purpose white grape cultivars.

Cultivar	Proportion of correct classification (%)		
	PsN	**BnC**	**Total**
PsN	**100.0 (599)**†	**–**	100.0 (599)
BnC	–	**100.0 (300)**	100.0 (300)
Overall	–	–	**100.0 (899)**

The correct classification performance is highlighted in bold. †Number of seeds analysed. BnC, Bianco di Chilivri; PsN, Pascale di Nurri.

The 100% correct identification when comparing the two dual-purpose white grape cultivars (Table 5) demonstrates that Pascale di Nurri (PsN) and Bianca di Chilivri (BnC) have little morphological similarity. No comparison among dual-purpose black berry cultivars was possible, as there was only one cultivar in this category.

A statistical comparison among the black winegrape cultivars was implemented in order to recognise possible synonymy groups (Table S1). The evaluation of the performance of each cultivar, and above all the assessment of the relative mistakes, allowed some assemblages to be formulated. Falso Gregu (FlG), Nasco nero di Abbasanta (NsA), Nera tomentosa (NeT), Nieddu mannu di Nurri (NNr), Nieddu mannu di Pattada (NPt) and Nieddu Pedra Serra (NPS) could be grouped into a unique synonymy group, confirming and building on the work of De Mattia et al. (2007) and Orrù et al. (2012). Similar evaluations allowed a few other possible synonymy groups to be formulated. Aniga di Lanusei (AnL), Cannonau (Cn), Cannonau nero di Sestu (CnS) and Nera liscia di Montresta (NeL) could be considered as belonging to the same synonymy group, as well as Cagnulari di Nurri (CgN), Canulare di Triei (CrT) and Falso Canulare di Triei (FlC). Also, the cultivars Carenisca (Cs), Muristellu (Mu) and Muristeddu di Sorgono (MuS) appear to belong to the same group, just as Gregu Nieddu del Campidano (GNC), Gregu Nieddu di Serramanna (GNS) and Vertudi (Vrt), misattributions of which are crossed. The evaluation of the results of this preliminary comparison allowed the possibility that many other new small synonymy groups are in accord with those of De Mattia et al. (2007) and Orrù et al. (2012). Nera di Bosa (NeB) and Nieddu Polchinu (NPl) appear to be morphologically similar, not only to each other, but also to Cannonatu anticu di Bitti (CnB), as well as Monica di Escalaplano (ME) and Monica di Seulo (MSl), previously grouped by De Mattia et al. (2007) and Orrù et al. (2012), and can be enlarged with the cultivar Monica nera (MN). Other small similarity groups could be constituted by Fiudedda di Sini (FdS) and Teresina di Sini (TrS); Monica di Sorgono (MSr) and Nera di Abbasanta (NeA); Barbera Sarda (BrS) and Nera di Escalaplano (NeE); Nera di Sini (NeS) and Salude e passa di Serramanna (SpS); and finally Nera glabra di Modolo (NeM), Niedda carta di Seulo (NSl) and Nuragus nero di Sini (NrS) (Table S1).

Likewise, a comparison among the white winegrape cultivars was implemented to make easier the identification procedure of possible synonymy groups (Table S2). Nuragus (Nr), Nuragus Arrubiu (NrA), Nuragus Moscatello (Nrt),

Table 6. Proportion of correct classification among the dessert black winegrape cultivars.

Cultivar	Proportion of correct classification (%)							
	Gr	GrG	Grs	MnG	MrS	MnS	MnU	Total
Gr	**93.3 (1122)**†	1.6 (19)	0.1 (1)	1.0 (12)	1.1 (13)	2.0 (24)	1.0 (12)	100.0 (1203)
GrG	4.4 (26)	**80.9 (480)**	0.8 (5)	0.3 (2)	4.4 (26)	4.4 (26)	4.7 (28)	100.0 (593)
Grs	–	0.5 (3)	**87.1 (520)**	–	0.3 (2)	–	12.1 (72)	100.0 (597)
MnG	2.8 (34)	0.5 (6)	–	**85.3 (1022)**	0.8 (10)	10.5 (126)	–	100.0 (1198)
MrS	2.8 (17)	6.7 (40)	–	1.8 (11)	**86.5 (518)**	2.2 (13)	–	100.0 (599)
MnS	5.3 (32)	4.0 (24)	0.2 (1)	34.8 (209)	3.0 (18)	**52.7 (316)**	–	100.0 (600)
MnU	1.5 (9)	6.2 (37)	15.2 (91)	–	–	0.2 (1)	**77.0 (461)**	100.0 (599)
Overall								**82.4 (5389)**

The correct identification performance is highlighted in bold. †The number of seeds analysed (shown in parenthesis). Gr, Girò; GrG, Girò di Gonnos; Grs, Girò scuro di Serri; MnG, Moscatello nero di Genuri; MnS, Moscato nero di Sini; MnU, Moscato nero di Ulatirso; MrS, Moscatello rosso di Seulo.

Nuragus Muscadeddu (Nrd) and Nuragus rosso rompizzolla (NrR) could be considered as belonging to the same synonymy group, as well as Cannonau bianco di Trieri (CnT), Cannonau bianco di Oliena (CnO) and Sinnidanu (Sn), confirming the work of Orrù et al. (2012). Furthermore, the cultivars Albacanna di Triei (AbT), Calabresa di Seulo (ClS) and Panzale di Ula (PzU) appear to belong to the same group. Other small synonymy groups could be constituted by Bianca pelosa di Montresta (BnM) and Licronaxiu (Lx); Mizu (Mz) and Semidano (Sm); and Lacconarzu di Abbasanta (LcA) and Retagliaddu di Monti (RtM).

The dessert winegrape cultivars were compared to identify possible synonymy groups. From the evaluation of the proportion of misattribution achieved by the comparison among the dessert wine black cultivars in the study, it appears that two small groups could be formed: Girò scuro di Serri (Grs) and Moscato nero di Ulatirso (MnU) could be grouped in the same group, and Moscatello nero di Genuri (MnG) and Moscato nero di Sini (MnS) in another (Table 6).

Using the same statistical analysis and the same evaluation procedure of the identification proportion, two large groups were recognised among the dessert white wine cultivars (Table S3). Aregu biancu (ArB), Vernaccia di Escalaplano (VrE), Vernaccia di S. Rosalia (VrR), Vernaccia bidri di Villasor (VrV), Vernazza di Orosei (VrO), Granatza di Mamoiada (GzM) and Granatza Aregu di Seulo (GzS) could be considered as belonging to the same group, confirming and enhancing the synonymy group proposed by Orrù et al. (2012). Similarly, Moscato di Lodine (MLd), Moscato di Tempio 1 (MT1), Moscato su pinu di Mamoiada (MSP), Moscatello bianco (Mb), Moscato bianco di Genuri (MbG), Moscato bianco di Sini (MbS) and Moscato di Fonni (MFn) appear to form a unique group, corroborating and enhancing one of the groups proposed by Orrù et al. (2012). Two other smaller possible groups were identified among these cultivars: one constituted by Arvesiniadu (Av) and Alvaranzeniadu bianco di Bosa (AvB), and the other by Codronisca (Cd) and Nasco (Ns).

Following the implications of the classifiers and the suggestions of the historical and cultural information about the end use of the grapevine cultivars studied, on the basis of the achieved results, two further discrimination analyses were conducted, formulating some new synonymy groups. The analysed 54 black berry cultivars were grouped into 14 synonymy groups, leaving eight of them out as independent cultivars (Table S4), confirming the six groups (G1, G2, G3, G6, G8 and G14) proposed by Orrù et al. (2012). Similarly, the 61 white berry cultivars were grouped into 15 synonymy groups, leaving nine of them out as univocal cultivars (Table S5), confirming also in this case the six groups (G4, G5, G7, G9, G10 and G11) proposed by Orrù et al. (2012).

The black berry cultivars Nieddu mannu di Nurri (NNr) and Nasco nero di Abbasanta (NsA) were added to the G1 proposed by Orrù et al. (2012), and were considered synonyms of Falso Gregu (FlG), Nera tomentosa (NeT), Nieddu Pedra Serra (NPS), Nieddu mannu di Pattada (NPt) and Primidivu Nieddu (PrN), reaching the 67.5% correct identification (Table S4). Similarly, the cultivar Monica nera (MN) was added to the G2, assumed as synonymous with Monica di Escalaplano (ME) and Monica di Seulo (MSl). For this group, a performance of correct classification of 60.1% was achieved (Table S4). Adding the cultivar Nera di Abbasanta (NeA), the group G3 showed 65.7% correct detection (Table S4), although not all the cultivars analysed by Orrù et al. (2012) were available for this work. The black berry cultivar group G6 proposed by Orrù et al. (2012), exclusively constituted by Cannonau (Cn), was enlarged with the cultivars Aniga di Lanusei (AnL), Cannonau nero di Sestu (CnS), Girò scuro di Serri (Grs), Moscato nero di Ulatirso (MnU) and Nera liscia di Montresta (NeL), obtaining more than 85% correct identification (Table S4). Also, the group G8 was enlarged with a further cultivar, Cannonau anticu di Bitti (CnB), achieving a proportion of correct recognition of 55.6%; the group G14 remained unchanged, showing a correct identification of 64.9% (Table S4). The new hypothetical groups from G22 to G29 show a proportion of correct identification ranging between 54.7 (G22) and 73.8% (G27) (Table S4). In Table 7, the cultivars included in each synonymy group are reported. The cultivars Apesorgia nera (ApN), Axina de tres bias (Axt), Caricagiola di Monti (CcM), Girò (Gr), Girò morbido di Serri (Grm), Mustiosa (Ms), Ocre e boe di Orosei (ObO) and Nera di Orosei (NeO) were not grouped in any synonymy group, remaining independent cultivars and showing correct classification performance between 53.9% (Gr) and 89.7% (Ms). An overall cross-validated proportion of correct recognition of 67.9% was reached for the new hypothetical synonymy black cultivar groups (Table S4).

Of the white cultivar synonymy groups proposed in this work, the groups G4, G7 and G9 remained unchanged compared with the results obtained by Orrù et al. (2012), showing a

Table 7. New hypothetical synonymy groups achieved on the basis of morpho-colorimetric data.

New synonymy groups	Grape cultivar
G1	**Falso Gregu; Nera tomentosa**; Nieddu mannu di Nurri; **Nieddu Pedra Serra**; **Nieddu mannu di Pattada**; Nasco nero di Abbasanta; **Primidivu Nieddu**
G2	**Monica di Escalaplano**; Monica nera; **Monica di Seulo**
G3	**Monica di Sorgono**; Nera di Abbasanta
G4	**Nuragus**; **Nuragus Arrubiu**; **Nuragus Moscatello**; **Nuragus Muscadeddu**; **Nuragus rosso rompizzolla**
G5	**Aregu biancu**; Granatza Aregu di Seulo; Granatza di Mamoiada; Vernaccia bidri di Villasor; **Vernaccia di Escalaplano**; **Vernaccia di S. Rosalia**; Vernazza di Orosei
G6	Aniga di Lanusei; **Cannonau**; Cannonau nero di Sestu; Girò scuro di Serri; Moscato nero di Ulatirso; Nera liscia di Montresta
G7	**Cannonau bianco di Oliena**; **Cannonau bianco di Trieri**; **Sinnidanu**; **Vernaccia di Solarussa**
G8	**Nera di Bosa**; **Nieddu Polchino**; Cannonatu anticu di Bitti
G9	**Culupuntu**; **Moscato di Pattada**; **Moscato di Tempio 2**
G10	Moscatelo bianco; Moscato bianco di Sini; Moscato di Fonni; **Moscato di Lodine**; **Moscato di Tempio 1**; **Moscato su pinu di Mamoiada**
G11	Bianca di Lodine; **Gregu bianco**
G12	Codronisca; Nasco
G13	Cuccuau di Ula; Monica Bianca
G14	**Gregu Nieddu del Campidano**; **Gregu Nieddu di Serramanna**; **Vertudi**
G15	Mizu; Semidano
G16	Lacconarzu di Abbasanta; Retagliaddu di Monti
G17	Albacanna di Triei; Calabresa di Seulo; Corofulu di Oliena; Galoppu di Escalaplano – Galoppu di Nurri; Panzale di Ula; Tittibacchina di Mamoiada
G18	Luglienca di Abbasanta; Olopo di Triei
G19	Tittiacca di Gonnos; Tittiaca verde di Serramanna
G20	Alvaranzeniadu bianco di Bosa; Arvesiniadu
G21	Bianca di Chilivri; Bianca pelosa di Montresta; Bianca pelosa di Montresta; Licronaxiu
G22	Girò di Gonnos; Licronaxiu nero Nuraxinieddu
G23	Aghina'e cressia di Abbasanta; Nera di Sini; Salude e passa di Serramanna
G24	Barbera Sarda; Nera di Escalaplano
G25	Fiudedda di Sini; Teresina di Sini
G26	Carenisca; Muristeddu di Sorgono; Muristellu
G27	Cagnulari di Nurri; Canulare di Triei; Falso Canulare di Triei
G28	Moscatello nero di Genuri; Moscatello rosso di Seulo; Nera di Janna Ritha
G29	Moscatello rosso di Seulo; Nera glabra di Modolo; Niedda carta di Seulo; Nuragus nero di Sini

The groups previously proposed by Orrù et al. (2012) are confirmed and are shown in bold.

proportion of correct identification between 63.4 (G4) and 67.6% (G9) (Table S5). The group G5 was enlarged, adding the cultivars Granatza Aregu di Seulo (GzS), Granatza di Mamoiada (GzM), Vernaccia bidri di Villasor (VrV) and Vernazza di Orosei (VrO), as well as the group G10, which was enhanced with the addition of Moscatello bianco (Mb), Moscatello bianco di Sini (MbS) and Moscato di Fonni (MFn). These two groups achieved a proportion of correct recognition of 92.2 and 85.6%, respectively (Table S5). Also, the white cultivar group G11 proposed by Orrù et al. (2012), exclusively constituted by Gregu biancu (GB), was enlarged with the cultivar Bianca di Lodine (BnL), obtaining 52.8% of correct identification (Table S5). The new hypothetical groups G12 and G13 and from G15 to G21 show a proportion of correct identification ranging between 53.9 (G15) and 88.9% (G19) (Table S5). The cultivars Axina de Francia (AxF), Corniola bianca (Cob), Granazza di Garaumele (GzG), Remugiau di Serri (RmS), Aghina de Gerusalemme di Abbasanta (AGA), Alicante (Al), Bianca di Padria (BnP), Caddiu biancu di Serri (CbS) and Pascale di Nurri (PsN) did not show similarity with the proposed groups, remaining independent cultivars. These cultivars show a proportion of correct identification ranging between 50.5 (BnP) and 79.2% (GzG). The overall cross-validated proportion of correct classification was 72.3% for the new hypothetical synonymy white cultivar groups (Table S5).

Conclusions

From the historical point of view, the geographical position of Sardinia had a fundamental role to facilitate cultural exchanges, agronomic and technical knowledge, commercial contacts, and trade among local Nuragic and eastern and western civilisations during the millennia. On the other hand, as asserted by Ucchesu et al. (2014), the presence of numerous wild grape populations, which still remains unaltered due to the geological and climatic conditions of Sardinia, and the existence of pristine habitats in the island, suggest that Sardinia could be a valid site of secondary domestication of grapes. Consequently, it appears clear that Sardinian grapevine cultivars have arisen from different breeding events involving both imported and local material, as well as both domesticated and wild grapes. Despite the numerous studies conducted, however, many Sardinian cultivars are simply the result of linguistic distortions due to the wide historic-cultural heterogeneity of the Island, leading to a high diversity in grape names. This, combined with actual cultivar diversity, has led to the apparent unusually high diversity of grapevine 'cultivars' in Sardinia (De Mattia et al. 2007).

Image analysis technology has often been used to screen and compare different plant cultivars, allowing inferences to be made about morphological and genetical diversity, and to describe interrelationships among organisms at different taxonomical levels without the confounding environmental effects (Bacchetta et al. 2008a, 2010, Mattana et al. 2008, Grillo et al. 2010, 2012). The 54 black berry cultivars analysed in this study were grouped into 14 synonymy groups and eight independent cultivars, while the 61 white berry cultivars were grouped into 15 synonymy groups and nine independent cultivars. These results not only confirm the synonymy groups (G1, G2, G3, G6, G8, G14 for the black berry cultivars, and G4, G5, G7, G9, G10, G11 for the white berry cultivars) already proposed by Orrù et al. (2012), but also show an increase in the number of cultivars within each group. Moreover, the increased number of accessions analysed resulted in an increase in the number of the identified synonymy groups, suggesting that more research should be undertaken on native cultivars, for example their molecular characterisation with a higher number of microsatellite markers. It is possible that the same cultivars, cropped in different times and/or in different areas, and probably applying different cultural practices, can show variable berry morphology. This could have been the cause of different typical end use in different regional areas. Consequently, this should explain some apparent incongruences, revealed in this work, related to the consumption purpose of some cultivars belonging to the same synonymy groups. The results achieved appear to be consistent with the geographical distribution of the grapevine cultivar and with the historical and cultural knowledge, and are in agreement with the results of Orrù et al. (2012).

It has been possible again to prove the effectiveness of the seed biometric features to characterise, identify and compare seed groups. As shown by much previous research (Smykalova et al. 2011, 2013, Pinna et al. 2014, Santo et al. 2014), the method can easily be applied to many other species, interesting from the agronomical and botanical point of view, as well as to any other geographical area, more or less extended or isolated.

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Table S1. Correct classification proportion among the analysed black grapevine cultivars.

Table S2. Correct classification proportion among the analysed white winegrape cultivars.

Table S3. Correct classification proportion among the analysed dessert white winegrape cultivars.

Table S4. Correct classification proportion among the new hypothetical synonymy black cultivar groups.

Table S5. Correct classification proportion among the new hypothetical synonymy white cultivar groups.