

## Genetic relationships between Sardinian and Spanish viticulture: the case of ‘Cannonau’ and ‘Garnacha’

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### SUMMARY

To evaluate the relationship between Sardinian and Spanish viticulture, Simple Sequence Repeat (SSR) markers were applied to define the genetic profiles of 29 cultivated and 48 wild grapevine (*Vitis vinifera* L.) accessions. SSR data confirmed synonymy between ‘Cannonau’ and several Spanish accessions of ‘Garnacha Tinta’. SSR analysis also suggested that the ‘Garnacha’ group consisted of a heterogeneous pool of cultivars displaying different morphological and genetic traits (Link coefficient = approx. 0.5), probably caused by somatic mutation or accidental breeding events between closely-related grapevine accessions. In contrast, the ‘Vernaccia’ - ‘Granaccia’ Sardinian group was different from ‘Cannonau’ (Link coefficient = 0.8) and all Spanish ‘Garnacha Tinta’ and ‘Blanca’ accessions analysed. To understand the ‘Cannonau’ - ‘Garnacha’ relationship, we studied the origin of these accessions and their relationships with spontaneous wild grapevine. Both cultivars are ancient grapes that have been cultivated for many centuries in both Sardinia and Spain. Although the name ‘Garnacha’ may derive from the Italian word ‘Vernaccia’, molecular analysis excluded any direct genetic origin of the Spanish ‘Garnacha’, or Sardinian ‘Cannonau’ from the ‘Vernaccia’ - ‘Granaccia’ Sardinian group. Structure analysis split the samples analysed into three clusters (K = 3). The first two clusters corresponded to the cultivated samples, while the wild accessions were in the third cluster. Based on this information, we can exclude any direct origin of the ‘Cannonau’ - ‘Garnacha’ group from the wild grapevines analysed and distributed on Sardinia.

Sardinia is an Italian island located in the western Mediterranean to the south of Corsica between Italy, Spain, and Tunisia. Historically, it therefore occupied a strategic trading position in the Mediterranean. Much of the island is ideally suited to pasture and agriculture. *Vitis vinifera* L. is one of the most important species grown on the island, both as a spontaneous wild plant (*V. vinifera* subsp. *silvestris*) and as a cultivated crop (*V. vinifera* subsp. *vinifera*). The large number of grapevine cultivars recorded (Calò *et al.*, 1990; De Mattia *et al.*, 2007) are a result of the complex history of Sardinia which was colonised by many different populations. In 238 BC, the island became part of the Roman Empire. It was later invaded by various Mediterranean civilisations, including Spain. Sardinia was a Spanish colony from 1479 to 1714 and, during this period, commercial and cultural exchanges between the two regions resulted in modifications to its agricultural products. In the case of viticulture, it is notable that Spanish and Sardinian grapevine varieties often have similar-sounding names; for example, ‘Bobal’ and ‘Bovale’, ‘Cariñena’ and ‘Carignano’, and ‘Garnacha’, ‘Granaccia’ and ‘Granazza’. We assume that invaders influenced Sardinian viticulture by introducing new varieties (De Mattia *et al.*, 2007), improving the genetic

composition of the local germplasm. At the same time, certain Sardinian cultivars, some derived from the domestication and cultivation of wild local grapevines, could have been introduced into Spain.

The cultivar ‘Cannonau’ is one of the most important black grape varieties cultivated in Sardinia. Based on ampelographic and historical information, ‘Cannonau’ is considered to be a synonym of several Spanish red cultivars such as ‘Garnacha Tinta’, ‘Garnacho’, ‘Tinto Aragón’, ‘Alicante’, and ‘Garnatxa Negra’ (Calò *et al.*, 1990; Peñín *et al.*, 1997). In Spain, there are different accessions of ‘Garnacha’, including a white cultivar ‘Garnacha Blanca’ distributed in different Spanish regions. Identification of ‘Garnacha’ and its synonyms is further complicated by the high level of morphological variation found among plants cultivated under this name (Cabezas *et al.*, 2003). Besides the supposed synonymy with ‘Cannonau’, some Spanish researchers (Martinez *et al.*, 2006) used ampelographical techniques to suggest a relationship between ‘Garnacha’ and ‘Mencia’. In France, the USA, and Australia, ‘Garnacha Tinta’ is called ‘Grenache’ (Dry, 2004).

Based on these considerations, the first aim of our work was to define synonyms and false homonyms of ‘Cannonau’ and ‘Garnacha’ using DNA molecular markers (Thomas and Scott, 1993; Bowers *et al.*, 1996; Sefc *et al.*, 1999; Reale *et al.*, 2006). The second aim was

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to analyse the origins of accessions of 'Cannonau' and 'Garnacha' by studying their genetic relationships with wild grapevines in Sardinia.

## MATERIALS AND METHODS

### Plant material and morphological characterisation

The grapevine cultivars used in this study are listed in Table I. These consisted of 29 accessions cultivated in Sardinia (n = 14) and in different regions of Spain (n = 15). Table I gives the name of the variety, its abbreviation, berry colour, area of cultivation, and the germplasm collection used for each accession. Grapevines are propagated by cuttings, so, except for somatic mutation, all vines of one cultivar are genetically identical to one another (Botta *et al.*, 1995); thus, only one individual was analysed as being representative of each variety.

In the case of wild grapevine, 48 accessions were analysed. Considering that wild grapevine is dioecious, while cultivated grapevines are hermaphrodite, the mating system was used to discriminate wild from cultivated grapevines during sampling. All the wild populations were collected in areas of Sardinia with distinctive wild grapevine habitats; for example, wetlands and forests with high humidity and with trees on which wild grapevines grow as lianas. The reference codes and geographical locations of each wild accession are shown in Table II and Figure 1, respectively.

### DNA extraction and SSR analysis

Genomic DNA was extracted from young leaflets as described by Labra *et al.* (2001). Each sample was genotyped at 13 microsatellite loci: VVS2 (Thomas and Scott, 1993), VVMD5, VVMD7 (Bowers *et al.*, 1996), VVMD21, VVMD24, VVMD25, VVMD27 (Bowers

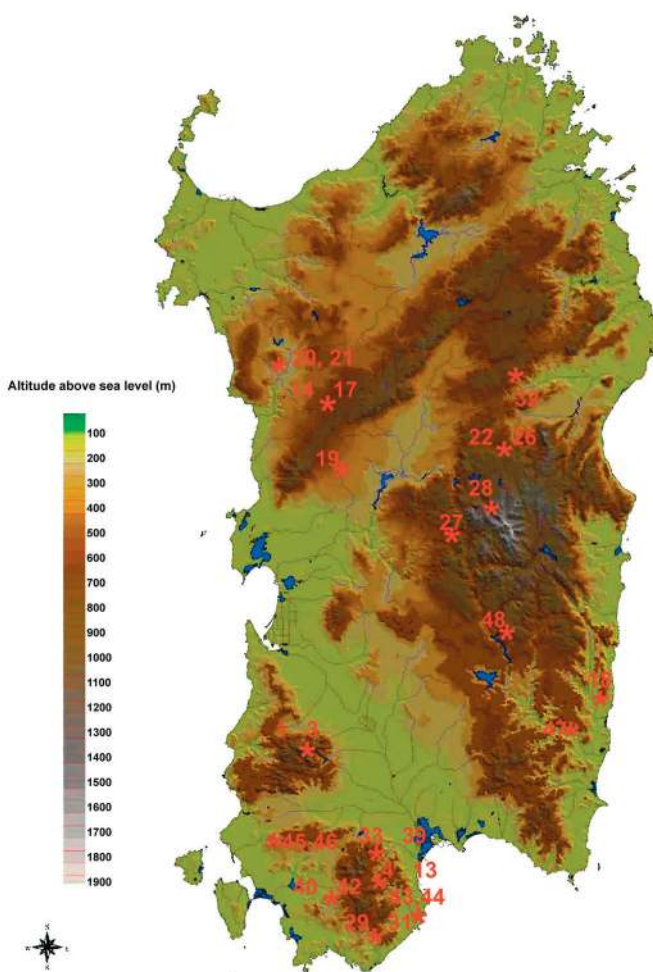


FIG. 1  
Map of Sardinia (Scale = 1:750,000) showing the geographical distribution of the 48 wild grapevine accessions sampled.

TABLE I  
The 29 grapevine cultivars analysed in this study

Cultivar Name	Berry colour	Cultivation area	Germplasm collection <sup>†</sup>	Abbreviation/Code
'Cannonau'	Black	Mandrolisai - Central Sardinia	AGRIS	CAN 1
'Cannonau'	Black	Sardinia - Cagliari	AGRIS	CAN 2
'Cannonau Bianco'	White	Triei, Ogliastra - Central Sardinia	AGRIS	CANB 1
'Cannonau Bianco'	White	Oliena Barbagia - Central Sardinia	AGRIS	CANB 2
'Cannonau'	Black	Villasor Campidano - Southern Sardinia	AGRIS	CAN 3
'Cannonau'	Black	Campidano - Southern Sardinia	AGRIS	CAN 4
'Tocai Rosso'	Black	Veneto - Northern Italy	ERSAF	TOC
'Garnacha Blanca'	White	Catalonia - North-eastern Spain	INCAVI	GB
'Garnacha Tinta'	Black	Catalonia - North-eastern Spain	INCAVI	GT 1
'Garnacha Tinta'	Black	Navarra - Northern Spain	EVENA	GT 12
'Garnacha Tinta'	Black	Aragon - North-eastern Spain	Diputación General de Aragón	GT 16
'Garnacha Tinta'	Black	Aragon - North-eastern Spain	Diputación General de Aragón	GT 20
'Garnacha Tinta'	Black	Aragon - North-eastern Spain	Diputación General de Aragón	GT 26
'Garnacha Tinta'	Black	Aragon - North-eastern Spain	Diputación General de Aragón	GT 30
'Garnacha Falsa'	Black	Aragon - North-eastern Spain	Diputación General de Aragón	GT 36
'Garnacha Tinta'	Black	Galicia - North-western Spain	Valdeorras Appellation	GT 3
'Garnacha Tinta'	Black	La Rioja - Northern Spain	CIDA La Rioja	GT 7
'Mencia'	Black	Galicia - North-western Spain	Valdeorras Appellation	ME 2
'Mencia'	Black	Galicia - North-western Spain	Ribeira Sacra Appellation	ME 5
'Mencia'	Black	Castilla y Leon - North-western Spain	ITACYL	ME 7
'Garnacha Tinta'	Black	Castilla-La Mancha - Central Spain	Mentrida Appellation	GT 98
'Garnacha Tinta'	Black	Castilla-La Mancha - Central Spain	Mentrida Appellation	GT 99
'Granaccia'	White	Oliena - Barbagia - Central Sardinia	AGRIS	GRAC
'Granazza'	White	Mamoiada - Barbagia - Central Sardinia	AGRIS	GRAZ
'Vernaccia'	White	Escalaplano, Ogliastra - Central Sardinia	AGRIS	VER 1
'Vernaccia'	White	Oristano, Ogliastra - Central Sardinia	AGRIS	VER 2
'Vernaccia di S. Rosalia'	White	Triei, Ogliastra - Central Sardinia	AGRIS	VER 3
'Vernaccia'	White	Solarussa, Campidano - Central Sardinia	AGRIS	VER 4
'Vernaccia'	White	Campidano - Southern Sardinia	AGRIS	VER 5

<sup>†</sup>ERSAF: Ente Regionale per i Servizi all'Agricoltura e alle Foreste, Lombardy, Italy; AGRIS: Agricultural Research Agency of Sardinia; INCAVI: Institut Català de la Vinya i el Vi, Spain; EVENA: Estación de Viticultura y Enología de Navarra, Spain; CIDA: Centro de Investigación y Desarrollo Agrario de La Rioja, Spain; ITACYL: Instituto Tecnológico Agrario de Castilla y León, Spain.



origin, or to more than one population. In this study, STRUCTURE 2.1 was used with the admixture model (i.e., each individual drawing some fraction of its genome from each of the K populations) and the frequencies-independent option (i.e., allele frequencies in one population are not necessarily related to allele frequencies in another).

Burn-in length was fixed to 100,000, following the suggestions of Pritchard and Wen (2003). Since, the initial samples are not completely valid because the MCMC (Markov Chain Monte Carlo) has not stabilised, the burn-in samples allow you to discard these samples. After different trials in which we looked for MCMC convergence and consistency among runs with identical parameter values, an MCMC length of 1,000,000 iterations seemed the most suitable.

Because such long runs are time-consuming, two types of run were performed because we suspected the presence of at least two groups. For K = 1 to 4, we performed six long runs per value of K, with MCMC lengths of 1,000,000 iterations; and, for K = 5 to 8, three short runs were performed per K, each with 500,000 iterations.

We selected the K values showing the optimal subdivision of the data, using the formula (Garnier *et al.*, 2004):

$$[Ln P(D) | k - Ln P(D) | k - 1]$$

where  $Ln P(D)$  was the estimated posterior probability of the data conditional to K.

At every iteration of the algorithm, the probability of each individual belonging to any of the different categories was computed, given the current values of the allele frequencies and the mixing proportions.

## RESULTS

### Synonyms and false homonyms

A total of 77 individual plants were analysed using SSR markers. Thirteen microsatellite loci were studied, and a total of 99 alleles were detected. The maximum number of observed alleles was 11 for VVMD7, while the minimum was six for each of the loci VVMD21, VVMD24, VVMD25, and ZAG47. Table II and Table III show the allele profiles detected for each wild and cultivated *Vitis* accession, respectively.

To define the relationships between 'Cannonau', 'Garnacha', and synonymous-homonymous varieties, the genetic profile of each cultivar was used to compute similarity matrices. A UPGMA dendrogram based on this matrix is shown in Figure 2. The data suggest that the four black 'Cannonau' varieties collected at different sites in Sardinia showed complete genetic identity. Some Spanish accessions of 'Garnacha Tinta' from different areas (codes GT 1, 7, 12, 30, 98, and 99) showed the same SSR profile as 'Cannonau' (Table III). This confirmed the synonymy between 'Cannonau' and several Spanish accessions of 'Garnacha Tinta'. However, other 'Garnacha Tinta' cultivars (codes GT 3, 16, 20, 26, and 36) showed a different genetic constitution to 'Cannonau'. The 'Garnacha Blanca' (GB) and 'Cannonau Bianco' (CANB 1 and 2) cultivars exhibited distinctive SSR profiles. An interesting similarity was observed between 'Cannonau' (codes CAN 1, 2, 3, and 4) and 'Cannonau Bianco' (codes CANB 1 and 2) that shared 50% of SSR alleles, suggesting a direct genetic relationship.

The three 'Mencia' cultivars showed the same SSR profile, but were different from all 'Garnacha' and 'Cannonau' samples analysed (Figure 2; Table III). Our results indicate that 'Mencia' is not related to 'Garnacha Tinta'.

TABLE III  
Genetic profiles of 29 grapevine cultivars analysed at 13 SSR loci

Cultivar	SSR marker													Group
	VVS2	VVMD5	VVMD7	VVMD21	VVMD24	VVMD25	VVMD27	VVMD28	ZAG21	ZAG47	ZAG62	ZAG64	ZAG79	
CAN 1	137-145 <sup>†</sup>	226-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
CAN 2	137-145	226-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
CANB 1	135-137	240-240	244-244	245-245	205-213	238-252	189-193	244-258	198-200	165-170	188-188	136-136	244-258	1
CANB 2	135-137	240-240	244-244	245-245	205-213	238-252	189-193	244-258	198-200	165-170	188-188	136-136	244-258	1
CAN 3	137-145	226-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
CAN 4	137-145	226-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
TOC	137-145	226-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
GB	137-145	226-240	240-244	239-245	207-213	238-252	195-195	244-244	200-202	170-170	188-188	136-142	258-258	1
GT 1	137-145	226-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
GT 12	137-145	226-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
GT 16	137-145	228-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
GT 20	137-145	226-240	240-244	239-251	205-213	238-246	193-193	244-244	200-202	157-170	188-188	136-142	258-258	1
GT 26	137-145	232-238	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
GT 3	133-145	226-238	240-244	239-245	205-207	238-238	181-189	244-260	198-202	157-170	188-188	136-159	244-258	1
GT 30	137-145	226-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
GT 36	137-145	228-234	240-244	245-251	205-207	238-252	185-193	236-244	200-204	161-170	188-188	136-142	248-258	1
GT 7	137-145	226-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
ME 2	145-151	226-236	250-258	239-251	205-205	238-246	181-189	236-236	200-202	157-165	188-194	136-138	248-252	2
ME 5	145-151	226-236	250-258	239-251	205-205	238-246	181-189	236-236	200-202	157-165	188-194	136-138	248-252	2
ME 7	145-151	226-236	250-258	239-251	205-205	238-246	181-189	236-236	200-202	157-165	188-194	136-138	248-252	2
GT 98	137-145	226-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
GT 99	137-145	226-240	240-244	239-245	207-213	238-252	193-193	244-244	200-202	170-170	188-188	136-142	258-258	1
GRAC	143-151	226-240	248-248	239-251	205-205	252-252	179-179	228-248	200-202	155-155	194-204	140-194	252-260	2
GRAZ	143-151	226-240	248-248	239-251	205-205	252-252	179-179	228-248	200-202	155-155	194-204	140-194	252-260	2
VER 1	143-151	226-240	248-248	245-245	205-205	238-260	179-179	228-248	202-204	155-157	194-204	136-159	252-260	2
VER 2	143-151	226-240	248-248	239-251	205-205	252-252	179-179	228-248	200-202	155-155	194-204	140-194	252-260	2
VER 3	143-151	226-240	248-248	239-251	205-205	252-252	179-179	228-248	200-202	155-155	194-204	140-194	252-260	2
VER 4	133-155	232-246	250-264	239-245	205-213	238-246	181-193	234-236	188-188	157-170	194-200	148-159	252-260	2
VER 5	133-143	228-232	248-254	239-245	213-213	238-252	185-185	244-258	192-200	161-161	194-200	136-163	240-252	2

<sup>†</sup> Allele sizes are given in base pairs.

The last column (Group) indicates the result of the structural analysis. Table I describes the characteristics of each accession.

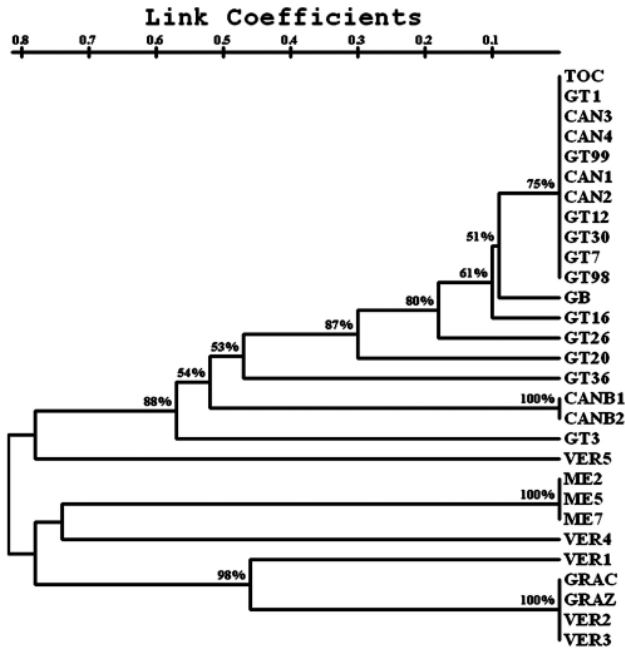


FIG. 2

Dendrogram based on Link coefficient index (Link *et al.*, 1995), showing genetic relationships between ‘Cannonau’ and related Sardinian and Spanish cultivars of *V. vinifera* L. Bootstrap values at branch point were computed using 2,000 replicates. See Table I for cultivar codes.

Finally, the comparison between Sardinian ‘Cannonau’ and the ‘Vernaccia’-‘Granaccia’ group (codes VER 1, 2, 3, 4, 5, GRAC, and GRAZ) indicated that these varieties are very different to ‘Cannonau’ and also to all the Spanish ‘Garnacha Tinta’ and ‘Garnacha Blanca’ analysed. Although this group consisted of cultivars with common morphological traits (i.e., white berry colour), only some accessions showed the same genetic constitution (e.g., codes VER 2, 3, GRAC, and GRAZ).

*Genetic relationship between wild and cultivated accessions of Vitis.*

Table II shows the SSR allele profiles detected for all the wild grapevine samples. Generally, a high level of genetic difference was observed. It is proposed that this genetic variability correlates with the mating system of these dioecious, outbreeding *Vitis* sub-species. Structure analysis performed to define genetic relationships between wild Sardinian grapevines and the cultivated accessions, showed that  $Ln P(D)$  values increased sharply with  $K$  from 1 to 3, before reaching a plateau (Figure 3A). This suggests that the analysed accessions should be split into three groups ( $K = 3$ ). The first two groups corresponded to the cultivated samples (Table III), while the wild accessions were clustered in the third group (Figure 3B). With the exception of two cultivars in group 2 (VER 4 and VER 5), all the analysed accessions displayed characteristic and distinct SSR profiles of each group.

**A**

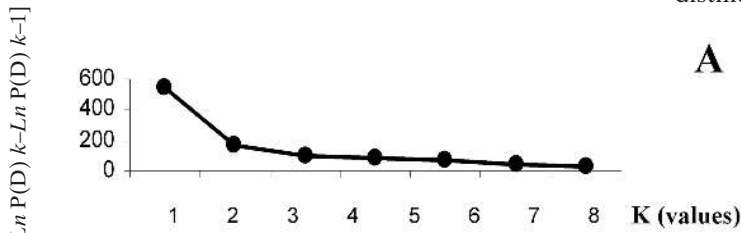
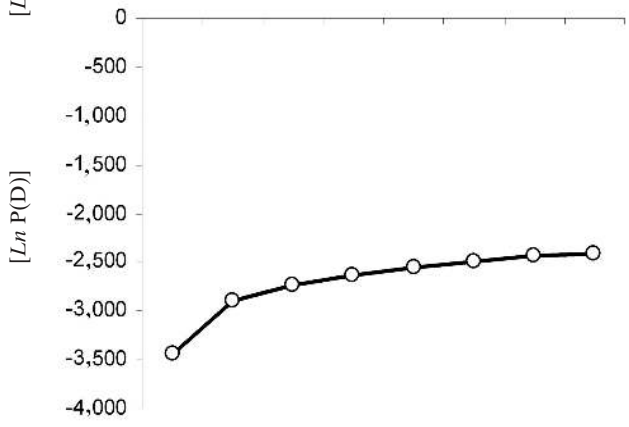
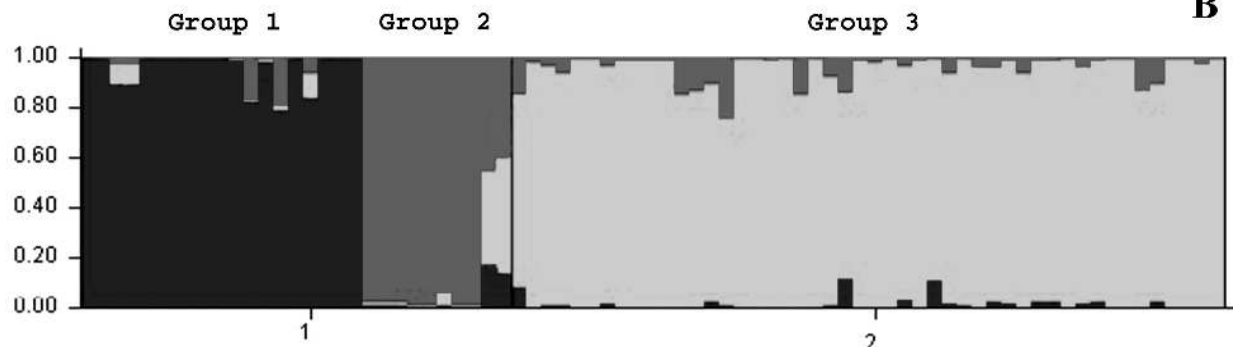


FIG. 3

STRUCTURE analysis data conducted on the SSR dataset. Panel A, the posterior probability of the data  $[Ln P(D)]$  against the number of  $K$  clusters (lower sub-Panel) and the increase of  $Ln P(D)$  given  $K$ , calculated as  $[Ln P(D)]k - Ln P(D)k-1$  (upper sub-Panel). The results suggest that  $Ln P(D)$  reaches a plateau with  $K = 3$ , thus the samples analysed should be split into three groups. Panel B, Bar plot of the results obtained from STRUCTURE using  $K = 3$ . Each individual is represented as a vertical line (total length of each bar represents a probability of 1.0) partitioned into three grey-scale segments whose lengths are proportional to the individual coefficients of membership in the three groups. Cultivars were present in groups 1 and 2, while wild accessions are shown in group 3 (see Table III).



**B**



Structure analysis also confirmed the common genetic constitution of the ‘Cannonau’ - ‘Garnacha’ accessions as suggested by the dendrogram in Figure 2, and their clear genetic difference from the other cultivated accessions.

The admixture proportions for  $K > 4$  did not reveal any new genetic structure and are not presented here.

## DISCUSSION

This study addressed the origin and diffusion of the grapevine cultivars ‘Cannonau’ and ‘Garnacha’ by studying their relationships with other grapevine accessions and an analysis of the viticultural history of Sardinia and Spain. The dendrogram based on SSR data (Figure 2) confirmed the synonymy between ‘Cannonau’ and several ‘Garnacha Tinta’ accessions. This molecular evidence was also supported by ampelographic information (Calò *et al.*, 1990; Cabezas *et al.*, 2003) and common morphological traits (data not shown).

Molecular analyses emphasised the genetic variation between members of the ‘Garnacha’ group. This agreed with data obtained by Cabezas *et al.* (2003) based on molecular and morphological research. Thus, the ‘Garnacha’ group consisted of a heterogeneous pool of cultivars, different in berry colour or in the presence of leaf hairs. In some cases, these morphological variations must have resulted from somatic mutations (Sevc *et al.*, 1998; Martin *et al.*, 2003); otherwise, the morphological differences could have been derived from genetic variations due to accidental breeding events between ‘Garnacha’ and other grapevine accessions. Based on these considerations, our results indicate that the synonymy between ‘Cannonau’ and ‘Garnacha Tinta’ was confirmed for only some of the ‘Garnacha Tinta’ accessions analysed.

To understand the origin of the ‘Cannonau’ - ‘Garnacha Tinta’ synonymy in more detail, we analysed the history of viticulture using different approaches. The first approach was to analyse the origin of the name “Garnacha”, which could derive from the Italian word “Vernaccia” (Corominas, 1967). “Vernaccia” indicates a white wine spread throughout Europe during and after the middle ages (Henry, 1986). Nowadays, the Spanish word “Garnacha” indicates a black or white variety (called ‘Garnacha Tinta’ or ‘Garnacha Blanca’, respectively). The names “Vernaccia”, “Granazza”, or “Granaccia” are similar to ‘Garnacha’; however, our molecular analyses excluded any genetic relationship between the Spanish ‘Garnacha’ cultivars and synonymous ‘Vernaccia’ - ‘Granazza’ - ‘Granaccia’ accessions distributed around Sardinia. The first written evidence of ‘Garnacha’ in Spain is in a book entitled “Agricultura General” by Alonso de Herrera (1513). However, Alonso de Herrera’s description is vague and insufficient to support the conclusion that he described ‘Garnacha’ - ‘Cannonau’. In Sardinia, the first documented reference to ‘Cannonau’ dates back to 1549 (Cherchi Paba, 1977). These historical documents suggest that ‘Garnacha’ and ‘Cannonau’ are extremely ancient varieties that were cultivated for many

centuries in both Sardinia and Spain. However, this historical information does not support the autochthonous origin of these cultivars through direct domestication in their regions of spread.

Recent molecular analysis has revealed that several cultivars from the Iberian Peninsula display DNA chlorotypes that are compatible only with their having been derived from local wild grapevine populations (Grassi *et al.*, 2003; Arroyo-Garcia *et al.*, 2006). However, so far, no direct domestication events have been clearly demonstrated. Sardinia was a target site for secondary domestication events for several reasons: i) the island shows a large diffusion of wild grapevines distributed in humid areas; ii) grape pips from the late Bronze Age have been found at Sardinian Nuragic sites (Bakels, 2002), suggesting that wild grapes were used for wine production; and iii) previous molecular research on Sardinian grapevine germplasm supports secondary domestication events on the island (Grassi *et al.*, 2003). Based on these criteria, we analysed the genetic constitution of wild Sardinian grapevines and their relationships with certain cultivated varieties, including ‘Cannonau’. Structural analysis excluded the existence of any strict relationship between the two groups of Sardinian cultivars (groups 1 and 2) and the wild grapevines (group 3). Based on this information, we excluded any direct origin of ‘Cannonau’ from the analysed wild grapevines distributed on Sardinia, as well as from Spanish wild grapevine accessions, as suggested by previous researchers (Grassi *et al.*, 2003; Arroyo-Garcia *et al.*, 2006).

We concluded that the modern grapevines could have been derived from a long process of selection, followed by vegetative propagation. Genetic variations would have increased during this process as a result of somatic mutation as suggested for the ‘Garnacha’ accessions, or due to the occasional generation of spontaneous hybrids derived from crosses between cultivated and/or wild grapevines (as suggested for ‘Cannonau Bianco’ which shares 50% of its SSR with ‘Cannonau’). For these reasons, modern cultivated varieties have become genetically separated from wild varieties, as indicated by our structure analysis and as confirmed by previous research (Arroyo-Garcia *et al.*, 2006; Imazio *et al.*, 2006). This complicates any study of domestication events in grapevine and the identification of autochthonous cultivars from others arising from complex varietal exchanges during viticultural history.

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