Genet Resour Crop Evol
DOI 10.1007/s10722-016-0444-6

RESEARCH ARTICLE

Urgent need for preservation of grapevine (*Vitis vinifera* L. subsp. *vinifera*) germplasm from small circum-Sicilian islands as revealed by SSR markers and traditional use investigations

Alessandro Silvestre Gristina · Roberto De Michele · Giuseppe Garfì · Tommaso La Mantia · Ignazio Fontana · Patrizia Spinelli · Antonio Motisi · Francesco Carimi

Received: 6 April 2016 / Accepted: 23 August 2016

Abstract Since the last decades grapevine germplasm is undergoing a process of rapid genetic erosion. This process is of particular concern in minor circum-Sicilian islands, because of the sharp reduction of the cultivated surfaces and the shift of their economy from agriculture to tourism. Aiming at valorising and preserving the surviving varieties we collected 185 accessions during several surveys since 2007. Six nuclear microsatellite markers were used for germplasm characterization, yielding 75 different genetic profiles. We found out that most genetic profiles (39) were not listed in national and international grapevine databases, confirming that the Sicilian minor islands represent underexplored hotspots of genetic diversity for grapevine. We also identified several synonymies, often due to geographic isolation, having 20 varieties at least two names. Conversely, 18 homonyms collectively indicated 34 genetically different accessions. Interviews with farmers provided information on current and past usage, and the origin and type of cultivation practices as well. The study also shows the urgent need for preservation of local grapevine germplasm, due to the disappearance of the elder caretakers of these traditional varieties. For rare germplasm preservation most part of the collected grapevine cultivars were introduced in an ex situ collection field.

Keywords Biodiversity hotspot · Genetic erosion · Grapevine · Marginal cultivations · Microsatellites-simple sequence repeat (SSR) · Neglected cultivar · *Vitis vinifera*

Introduction

Traditional varieties and crop wild relatives represent an important biodiversity pool for both purely resources conservation goals and improvement of modern varieties (Fernie et al. 2006; Tanksley and McCouch 1997). In the last decades, genetic erosion affected many species of agronomical interest in the Mediterranean area (Hammer and Laghetti 2006). In industrialised countries, like Italy, the process of genetic erosion has been particularly rapid and is still ongoing. Such an impoverishment is mainly due to the collapse of traditional agricultural systems, with the degradation, fragmentation and loss of entire cultivated areas, following the abandonment of agricultural

Electronic supplementary material The online version of this article (doi:10.1007/s10722-016-0444-6) contains supplementary material, which is available to authorized users.

A. S. Gristina · R. De Michele · G. Garfì · I. Fontana · P. Spinelli · A. Motisi · F. Carimi (✉)
Istituto di Bioscienze e BioRisorse, UOS di Palermo, Consiglio Nazionale delle Ricerche, Corso Calatafimi 414, 90145 Palermo, Italy
e-mail: francesco.carimi@ibbr.cnr.it

T. La Mantia
Dipartimento SAF, dell'Università di Palermo, Viale delle Scienze 11, 90128 Palermo, Italy

Published online: 20 September 2016

activities (Hammer and Laghetti 2006). Relict traditional varieties only survive in marginal areas, such as small islands far away from the coast, that for their geographical isolation are generally richer in ancient or uncommon germplasm than the neighbouring continental areas (Hammer and Laghetti 2006).

In most of the minor circum-Sicilian islands, agriculture has been historically the major economic activity. Since the end of World War II, however, agriculture rapidly declined, and the economy became more and more dependent on tourism. Currently, marginal agriculture only survives thanks to a small number of aged farmers (La Mantia et al. 2011), suggesting that the traditional varieties and the local related knowledge will disappear in the next future.

Grapevine is one of the most important crops all around the Mediterranean Region and the Near East. Compared to the thousands of cultivars selected during its millenary history of domestication, clone selection, Phylloxera crisis and the massive diffusion of few international clones of varieties like Chardonnay and Cabernet Sauvignon caused a severe loss of diversity in many countries. Only in small islands and in the most remote inner areas, geographic isolation, peculiar edafo-climatic conditions and socio-economic reasons relatively limited this globalization trend, then involving the persistence of some traditional and/or ancient grape varieties. Sicily occupies a central position in the Mediterranean and has been historically a main junction of commerce and colonization, and the cradle for the development of many local varieties (Unwin 2005; Garfì et al. 2013). For these reasons, it can be expected that the circum-Sicilian archipelagos represent an interesting source of traditional varieties, worth to be saved from extinction and adequately valorised. In this study, we studied the genetic identity and traditional use of the grapevine germplasm of minor Sicilian islands. The goal was not only to promote the preservation of rare and unrecognized germplasm trough the introduction in an ex situ collection field, but also to treasure the historical knowledge associated with these varieties.

Materials and methods

Study area

Among the circum-Sicilian archipelagos, five areas were investigated: Salina (Aeolian Islands), Lampedusa and Linosa (Pelagie Islands), Pantelleria, and Ustica (Fig. 1). The Egadi Islands were not investigated because the grape cultivation has disappeared. A brief description of each island is reported hereinafter, while additional information is provided in Table 1.

Salina

Salina belongs to the Aeolian archipelago, NE of Sicily. It has volcanic origin and it emerged from the SE Tyrrhenian Sea about 0.3 million years ago (Lucchi et al. 2013). Its natural landscape is dominated by the steep cones of Monte dei Porri and Monte Fossa delle Felci: for this reason the ancient Greeks called it Didyme (= twin island). During the Middle Age, the population of Salina increased due to migrations from Lipari, the main island of the archipelago, which was experiencing intense volcanic activity. Among the Aeolian Islands, Salina has always been the most intensively cultivated. In the mid 1800 the viticulture was largely widespread and a number of varieties were cultivated (De Gregorio 1840). As for the whole Aeolian archipelago, Habsburg Lothringen (1894) reported that the most common grapes were the white *Cataratta* and *Malvasia,* and the black *Passulina*, *Mantuonica* and *Moscato*; specifically for Salina (in locality Santa Marina) the white cultivars *Nuciddara*, *Greca*, *Duraco*, *Ducignola*, and the black cultivars *Trummana* and *Livedda* were commonly used for trellis.

As in the rest of the Aeolian archipelago, the population dramatically dropped at the end of the nineteenth century, due to the huge destruction of vineyards caused by phylloxera (King and Young 1979; Lo Cascio and La Mantia 2013). Currently, the main crops are capers and grapes, the latter supporting a traditional wine industry (*Malvasia* wine, mostly). The local investigated vineyards are mainly located in the territories of Malfa and Val di Chiesa.

Lampedusa

Lampedusa belongs to the African–Pelagian foreland; local outcropping rocks are Meso-Cenozoic carbonates and marls (Grasso and Pedley 1988). It appears like a triangular plateau with an almost continuous steep cliff on the northern coast and gently declining slopes southwards, with several canyons; local

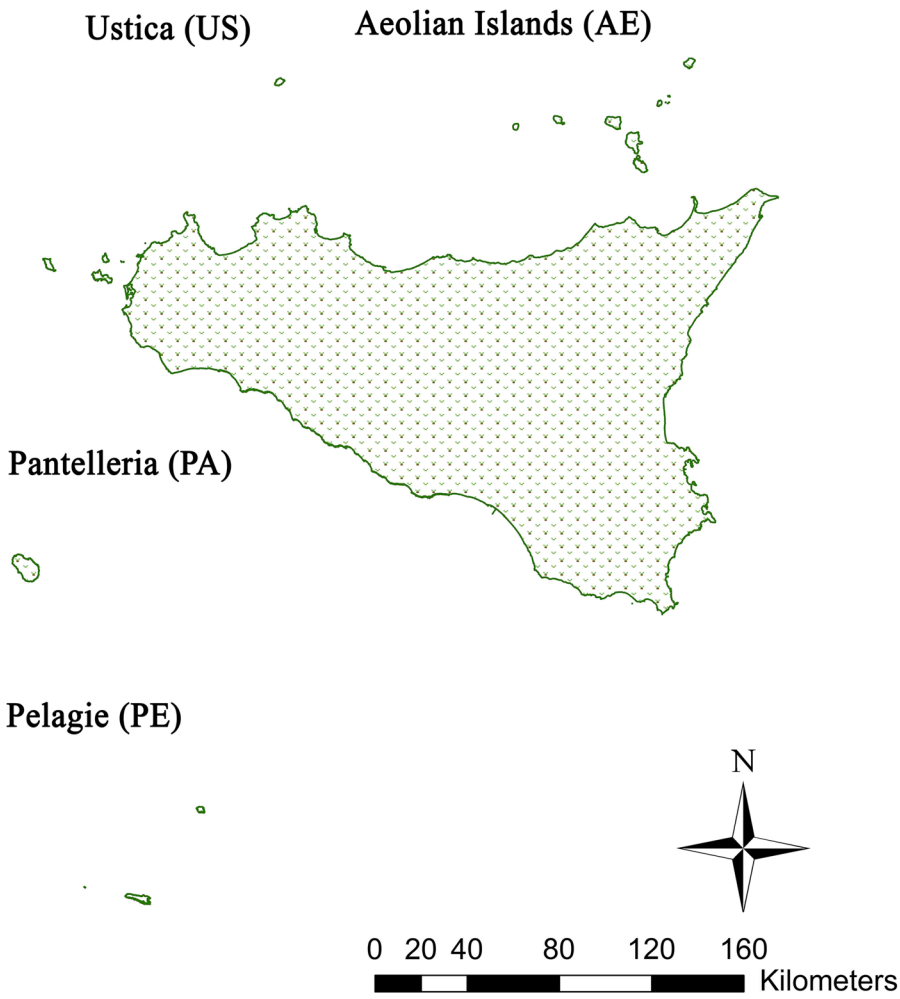


Fig. 1 Map of Sicily and circum-Sicilian archipelagos

agriculture developed inside these canyons, on intensively terraced surfaces (La Mantia et al. 2011). Lampedusa was first inhabited during the Neolithic and seems to have hosted a continuous human community until the end of 2000 BCE, probably related to the Maltese megalithic civilization (Radi 1973). Thanks to its wide natural harbour, it has been exploited as a naval base since ancient times and played a key role as stopover for North African, Maltese and Sicilian sailors and anglers over the centuries. After the establishment of a permanent Bourbon colony in 1843, grape cultivation was introduced in the island (Calcagno 1879) and until the end of the World War II Lampedusa was almost self-sufficient for agriculture. Today, only few cultivated fields, mostly vineyards (Di Lorenzo et al. 2010) and vegetable orchards, survive in very restricted areas (Hammer and Laghetti 2006; La Mantia et al. 2011).

Linosa

Linosa, a small volcanic island in the Strait of Sicily emerged between 1.1 and 0.5 million years ago (Di Bella et al. 2008). Archaeological remains testify its use as a base for Romans during the Punic Wars (fifth century BC). Like Lampedusa, after a long-lasting period of irregular human presence, it was colonized during the half of the nineteenth century (Corti et al. 2002). Agriculture and fishing, once the exclusive resources for local people, are currently in rapid decline. As observed in Pantelleria and the Aeolian islands, most of the cultivated terraces have been

Table 1 Synthetic overview on the main geographical and historical data of the investigated circum-Sicilian islands

Island	First settlements (k years BP)	Classical age (5th–3th BC)	Romans (3th–5th BC)	Byzantines (6th–9th)	Arabs (9th–11th)	Spaniards (13th–19th)	Max altitude (m a.s.l.)	Latitude	Longitude	Distance from the coast (km)	Surface (km^2)	Inhabitants (×1000)	Mean annual precipitations (mm)	Mean annual temperature (°C)
Salina	3.5	Greeks	X	X		X	962	38°33′49″	14°50′16″	38	26.4	2.3	615	18.0
Lampedusa	7	Greeks/Phoenicians	X	X	X	X	133	35°30′56″	12°34′23″	127	20.2	6.3	321	19.3
Linosa	2.4	Phoenicians	X			X	195	35°51′20″	12°51′70″	145	5.4	0.4	458	20.2
Pantelleria	>7	Phoenicians	X	X	X	X	836	36°47′27″	11°59′38″	67	82.9	7.7	466	18.0
Ustica	3.3	Phoenicians	X			X	239	38°42′48″	13°11′54″	52	8.3	1.3	450	16.9

abandoned and are nowadays colonized by natural vegetation due to progressive succession processes (Rühl and Pasta 2007).

Pantelleria

The volcanic complex of Pantelleria emerged about 0.3 million years ago (Civetta et al. 1984) between SW Sicily and Tunisia. It was first colonised during the Neolithic period (Abelli et al. 2014), and permanently inhabited since the eighth century BCE. Local dialect and toponyms largely testify the strong influence of Arab and Berber people on local culture and landscape shaping. Not surprisingly, the agricultural identity of Pantelleria is specially linked to the *Zibibbo*, a grape variety introduced from Cape Zebib (NE Tunisia) during the Arab domination (ninth–eleventh century CE) (Niccoli 1902), used to produce table grapes, sweet wine and raisin.

In the half of the nineteenth century Calcara (1853) documented a long list of grape varieties cultivated in the island for wine production, including *Cataratta* (*Cataratto), Greca di vigna, Blasco, Pignatello, Catalamiscu, Moscatello, Nano, Virduni, Uva di paradiso* and *Racina virdi*, in addition to *Zibibbo*, *Insolia* and *Bildè* used for both raisin and wine; as table grapes, the varieties *Greca, Caleo, Uva di Salemi, Prunesta, Minnavacchina bianca* and *nera, Buttuna di gallo* and *Trivolti* were also common. In 1833 vineyards extended over a total area of 1054 hectares, increasing to more than 3000 hectares at the beginning of the twentieth century (Scarponi 1939; Bonasera 1965). The two cultivars *Catarratto* and *Zibibbo*, used for common and sweet wine, respectively, have always been the prevailing grapes (Puviani 1916). Around the 1930, when the phylloxera outbreak largely spread in the island, farmers reacted expanding the cultivations in new terraced areas (Gigante 1968; D'Aietti 1978) and on the eve of World War II about 5000 hectares of these two varieties were still grown (Scarponi 1939). In the middle of the 1970, some additional grapes are reported, namely *Funcia chiatta, Inzolia, Minna i vacca, Nívuru, Pignatello* and *Greca* (D'Aietti 1978).

In the following decades, agriculture as a whole experienced a progressive decline and the Agricultural Usable Surface reduced by 60 % between 1929 and early 2000 (Rühl et al. 2005). Currently, the main agricultural products are grapes and capers (Hammer

and Laghetti 2006), grown on the few available flat areas (e.g. at Piana Ghirlanda) and on some terraced slopes in the localities of Mueggen, Bukkuram, Siba and Scauri.

Ustica

Ustica is a small volcanic island emerged about 0.3 million years ago in S Tyrrhenian Sea (De Vita et al. 1998). The island was first inhabited during the Eneolithic by peoples coming from the Aeolian Islands (Holloway and Lukesh 2001). Several centuries later, Phoenicians and Romans used it as a naval base, whilst during the Middle Age human presence started to be quite irregular and the island was totally deserted after the Thirteenth century. It became a hideout for North African pirates until the end of the eighteenth century, when a new Bourbon colony with people from Lipari was established and its natural landscape was rapidly transformed by agricultural activities. At that time, an area of about 350 hectares was devoted to viticulture and a number of black (e.g. *Muriedda*, *Vanni bertucci*, *Trummana/Tremani*, *Olivedda niura*, the latter known as an excellent table variety) and white (e.g. *Zibibbo*, *Muscateddu*, *Guarnacca*, *Rigalia*, *Lacrime i Madonna*, *Trunzu*, *Zuruca*, *Cornicchiola*) varieties were cultivated (Habsburg Lothringen 1898), especially in the gently declining slopes of the northern (Tramontana) and southern (San Paolo) sides of the east–west oriented small mountain ridge consisting of three extinct volcanoes.

Information and semi-structured interviews to farmers

In order to obtain information on grapevine germplasm, semi-structured interviews to farmers were carried out through a standardised questionnaire between 2006 and 2011. Forty-five informants (44 men and 1 woman aged from 41 to 94) were chosen with the assistance of local expert grapevine farmers. The majority of our informants were elders (55–94 years old, 62 %); for each of them personal data were noted, including gender, age, education and occupation. Farmers were asked to provide the following information: names and synonyms of grape varieties, berry colour, current and past usage, origin, time of introduction, type of management and growing practices. Information not related to traditional varieties was not recorded.

Plant material

The accessions were selected following the indications of the farmers and labelled in order to relocate the plants to collect plant material (leaves and young cuttings for DNA analyses and scions for grafting). Plant material was collected between 2007 and 2014 directly from 60 vineyards: 22 from Salina, 8 from Lampedusa, 6 from Linosa, 18 from Pantelleria and 6 from Ustica. The oldest vineyards (more than 110 years old) were located in Salina, while all the others were between 20 and 95 years old. Altogether 185 local cultivars were investigated by microsatellite analysis (Table 2). Out of these, 82 were collected in Salina, 34 in Lampedusa, 17 in Linosa, 39 in Pantelleria and 13 in Ustica. For rare germplasm preservation most part of the collected grapevine cultivars were introduced in an ex situ collection field of the National Research Council of Italy (CNR) Institute of Biosciences and BioResources (IBBR) located in Collesano district, Italy (37°59′19.9″N 13°54′55.8″E, 80 m above sea level).

DNA extraction and microsatellites analyses

Total genomic DNA was extracted from young leaves or inner wood of young cuttings. Tissues were ground into fine powder with liquid nitrogen and stored at −80 °C until use. The extraction was carried out following the CTAB method (Doyle and Doyle 1987) and DNA was quantified in 1 % agarose gels.

Samples were analysed at six microsatellite loci [Simple Sequence Repeat (SSR)], i.e. VVS2 (Thomas and Scott 1993), VVMD5, VVMD7 and VVMD27 (Bowers et al. 1996), VrZAG62, and VrZAG79 (Sefc et al. 1999). The forward primer of each marker was labeled with one of the three unique ABI PRISM fluorescent dyes: 6-FAM, JOE, TAMRA.

PCR amplification was carried out using the Qiagen multiplex PCR kit with the following conditions: 15 min at 95 °C (HotStar Taq activation step), followed by 35 cycles consisting of 30 s at 94 °C (denaturation), 90 s at 50–56 °C (annealing), 60 s at 72 °C (extension) and a final step for 30 min at 72 °C.

Each sample was amplified at least twice to correct for possible mistyping or amplification errors. PCR products were size-separated by capillary electrophoresis performed on a genetic analyzer (ABI

Table 2 List of sampled grapevine cultivars, sampling area, uses and field notes

	Cultivar local name	Sampling area[a] (no. of plants[b])	Berry colour	Use[c]	Field notes from the interviews
1	Albanella bianca	US (1)	White	W	
2	Albanello	US (1)	White	W	
3	Alicante	PA (2)	Black	W	Produces a wine with good acidity. The grape was probably introduced from Tunisia
4	Alivedda nera	US (1)	Black	T	
5	Aurora	US (1)	White	T	
6	Bertuccio	PE (4)	Black	T, W	A black table and wine grape with large berries. Native from Tunisia, it was introduced by Lampedusan sponge-divers during their long boat trips since the end of the 19th century
7	Calabrisi	PE (1)	Black	W	
8	Caleu	PA (1)	Black	T	
9	Cantaro	AE (1)	Black	W	Very productive. Its name derives from an old Sicilian measure of weight corresponding to 79 kg
10	Cappuccio	AE (1)	Black	W	
11	Catarratto	AE (2), PA (5), PE (1)	White	W	
12	Catarratto acino grosso	AE (1)	White	W	Big berry
13	Catarratto acino piccolo	AE (1)	White	W	Small berry
14	Catarratto di Pantelleria	PA (1)	White	W	
15	Catarratto rosato	PE (1)	Red	W	Pinkish berry
16	Centoruotoli, Centorotoli	AE (1)	Black	T, W	A black table and wine grape with small/medium berries is present in Salina. It is a reflowering variety. In certain years, grape ripening is incomplete. It was used in trellis. It confers acidity to wine when used with other varieties.
17	Centoruotoli, Centorotoli	PA (1)	White	T, W	A white table grape with small/medium berries. It is a reflowering variety. In certain years, grape ripening is incomplete. It was used in trellis. It confers acidity to wine when used with other varieties.
18	Citana	AE (2)	White	T	It is never used to make wine, since it converts into vinegar. The name Citana probably derives from *Acitu*, Sicilian word for vinegar
19	Corinto	AE (2)	Black	D, W	It is added to Malvasia in 5 % proportion to darken the must
20	Cornicchiola	AE (4)	White	T	Berry has the shape of little horn (*cornicchio* in Sicilian language)
21	Cuda i vulpe	AE (1)	White	W	It was used to make the Malvasia wine. It has been cultivated in Salina since ancient times, but it is native from the Island of Crete
22	Damaschino	US (2)	White	T, W	
23	Diretta	AE (1)	Black	W	Ungrafted varieties resistant to Phylloxera disease
24	Diretta bianca	AE (2)	White	T, W	
25	Diretta nera	AE (1)	Black	W	

Table 2 continued

	Cultivar local name	Sampling area[a] (no. of plants[b])	Berry colour	Use[c]	Field notes from the interviews
26	Fiore d'arancio	AE (3)	White	T, W	It is called "Fiore d'arancio" (Orange flower) in the village of Malfa, and "Trunzu" in the village of Santa Marina Salina
27	Funcia chiatta	PA (1), PE (5)	White	T	White table grape with large and disc-shaped berries, native from Tunisia. It was wrapped in white bags before harvesting. It is also conserved in alcohol
28	Gallipoli bianca	PE (2)	White	W	White grape from Apulia. A black version also exists, with small berries and dense bunch
29	Gallipoli nera	PE (2)	Black	W	Black grape from Apulia, with small berries and dense bunch
30	Garignano	PA (2)	Black	W	It was introduced from Tunisia at the beginning of 20th century. It is sensitive to oidium, therefore its diffusion is limited
31	Giugnatica	AE (1)	Red	T	Early grape that ripens in June
32	Greca	PA (2)	White	T, W	Table and wine grape, it is used in trellis
33	Inzolia	AE (2), PE (2)	White	T, W	
34	Inzolia imperiale	AE (1)	White	T, W	
35	Inzolia Linosana	PE (1)	White	W	More globose berry compared with typical elliptic Inzolia berries
36	Inzolia nera	AE (1), US (1)	Black	W	
37	Inzolia Tunisina	PA (2)	White	T	It was imported from Tunisia by Sicilian emigrants coming back home after the World War II
38	Lacrime i Madonna	PA (1), US (2)	White	T	Fruit are maintained by the plant until Christmas
39	Lacrime i Maria	AE (3)	White	T	It is reflowering. It is used in trellis
40	Livedda	AE (3)	Black	T	Its name derives from the shape of an olive. Crunchy fruit. Late ripening (October–November), it preserves until Christmas
41	Lugliatica, Luglienga	AE (1)	White	T	Early grape that ripens in July
42	Maddalena Salomone	PA (1)	White	T	Early ripening grape with berries of different size in the same bunch
43	Magliocco	AE (1)	Black	W	It grows well on dry-stone walls and is wind-tolerant
44	Malvasia	AE (7)	White	W	It is still used nowadays to make strong traditional sweet wine. Ripe grapes are collected and sun-dried for 2 weeks before wine-making
45	Mantonico	AE (2)	Red	T, W	Berries are very sweet and with soft flesh. Very productive. It was used to produce sweet wines
46	Maria Pirovano	PA (1)	White	T	
47	Mascarisi	AE (3)	Black	W	
48	Minna i vacca	PA (2), PE (3), US (1)	Red	T	It is used on trellis. It is native from Sfax (Tunisia). Berries are big and very sweet
49	Minna i vacca Linosana	PE (1)	Red	T	
50	Minnilottina	AE (2)	White	D, T	Table grape with soft skin. It is also used as raisin. Its internodes are long
51	Minnulettina	AE (1)	White	D, T	It is also used as raisin
52	Minutidda	AE (3)	Black	D, W	It is added to Malvasia in ca. 5 % proportion to darken the must

Table 2 continued

	Cultivar local name	Sampling area[a] (no. of plants[b])	Berry colour	Use[c]	Field notes from the interviews
53	Moscato nero	AE (2)	Black	T, W	
54	Nave	PA (2), PE (3)	White	T, W	White table grape, small and crunchy. Grape skin is thick. Bunches are big (up to 2.5 kg). It is well suited for being conserved in alcohol or wrapped in paper and hanged in the walls, until Christmas. It is native from Partinico (province Palermo-Sicily)
56	Nera da vino	PE (1)	Black	W	
55	Nerello Mascarisi	AE (1)	Black	W	
57	Nero d'Avola	PE (1)	Black	W	Imported from Marsala (Sicily)
58	Nivureddu	PE (4)	Black	W	It gives a dark hue to wines. In ancient times it was used to prepare the "Ribollito, Ribullito or Rimpollito" wine, an ink-hued very sweet wine, used during celebrations and to darken other wines, especially Catarratto. It was prepared by drying grapes in the sun for 15-16 days, isolating detached berries through a mesh (2 cm) before pressing. Resulting wine is sweet and with 16-17 % alcohol. The variety has been cultivated in Pantelleria since ancient times
59	Nivureddu acino grosso	PE (1)	Black	W	Big berries
60	Nivureddu acino piccolo	PE (1)	Black	W	Small berries
61	Nivureddu antico	PE (1)	Black	W	
62	Nivureddu arricciato	PE (1)	Black	W	Its name derives from the curly leaves
63	Nivureddu grosso	PE (1)	Black	W	
64	Nivureddu lasco	PE (1)	Black	W	Its name derives from the loose shape of the bunch
65	Nivuriddu	PE (1)	Black	W	It was imported from Tunisia by emigrants coming back home after the World War II
66	Nivuro/Nivuro nostrale/ Pignatello	PA (3)	Black	W	It was mixed with white grapes to produce a rosé wine
67	Nuciddara	AE (3)	White	D, T, W	Hard berry, it was used to make raisin
68	Paradiso	PE (2)	White	T, W	White wine and table grape, with perfectly round, sweet and tasty berry
69	Perricone	AE (1)	Black	T, W	
70	Pirricone	AE (1 type 1)	Black	W	There are three different types: 1) small berry with thick skin; 2) big berry, used also as table grape; 3) big berry and reddish stalk
71	Pirricone raspo rosso	AE (1)	Black	T, W	–
72	Pizzitello bianco	PA (1)	White	T	It was also called "*Cugghiuni 'i gaddu*" (Rooster's testicles)
73	Pizzutella bianca	PE (1)	White	T	
74	Putrisa	AE (1)	White	T	It is similar to Inzolia, but skin is thin and flesh is soft. It ripens at the beginning of August
75	Racina i mustu	AE (4)	Black	W	Ancient wine grape. Berry is small and crunchy. It is very productive. Native from Salina, it was considered the most important variety for making wine
76	Racina i ventu	AE (1)	White	W	There are white and black varieties. The black grape is similar to Inzolia Nera and gives a good, sweet wine. Its name (Grape of the wind) indicates that the berries easily fall when winds blow

Table 2 continued

	Cultivar local name	Sampling area[a] (no. of plants[b])	Berry colour	Use[c]	Field notes from the interviews
77	Regina	PA (1)	White	T	Early grape that ripens in June
78	Rucignola, Nucignola	AE (2)	White	W	Very sweet, wine and table grape. It was used to prepare the Malvasia wine, or to improve its alcohol content. Some berries in the bunch never fully ripen
79	Rusignola	AE (1)	White	W	
80	Sfaghesina	PE (1)	Red	T	It is native from Sfax (Tunisia), being brought to Lampedusa by the sponge collectors
81	Spogliammucca	PE (1)	Red	T, W	
82	Squagghiammucca	PE (2)	Red	T, W	Native from Tunisia
83	Trummana	AE (2), US (1)	Black	T, W	Black table grape, re-flowering, often used in trellis
84	Trunzu	AE (2)	White	T, W	Crunchy table grape. It is called Fiore d'arancio (Orange flower) in the village of Malfa, and Trunzu in Santa Marina
85	Vugliatico	AE (1)	Red	T	Early grape that ripens in July
86	Zibibbo	PA (6), PE (3), AE (1), US (1)	White	D, T, W	In Pantelleria island it is still used nowadays to make strong traditional sweet wine. Ripe grapes are collected and sun-dried for 2 weeks and later added to the must
87	Zibibbo antico	PE (2)	White	D, T, W	
88	Zibibbo bianco	AE (2)	White	D, T, W	
89	Zibibbo dorato	PA (1)	White	D, T, W	It is also called "Zibibbo Dorè (Golden Zibibbo)" or "Zibibbo giallo (yellow Zibibbo)". It is bright yellow even when not ripe. It ripens late. It originates from a mutant plant in the Ghirlanda area probably in the '80 s, and then it spread on the whole island
90	Zibibbo grappolo spargolo, Z. Masculone	PA (1)	White	D, T, W	Sweet grape, but with few berries on the bunch. Some farmers grow it because it is considered to be a good pollinator. It needs green pruning
91	Zibibbo minna i vacca	PA (1)	White	D, T, W	The berry has the shape of a pear
92	Zibibbo nero	AE (2), PA (1)	Red	D, T, W	
93	Zu Manuele	US (1)	White	W	
		Total 185			

[a] Sampling area: AE—Aeolian Islands (Salina), PA—Pantelleria Island, PE—Pelagie Islands (Lampedusa and Linosa), US—Ustica Island

[b] Enclosed within brackets, is reported the number of plants collected following farmer claims

[c] The use of the berries is indicated as: D—dried berries, T—table grape, W—wine grape

Prism 3130, Applied Biosystems, Inc.) by an external service (MWG, Germany).

Electropherograms were visually verified using Gene Mapper v. 4.1 software. Allele size was estimated by comparing the fragment peaks with the internal size standard, using the default method for band calling with SSR and the expected repeat size. Genetic profiles were compared with the six SSR-markers used within the Genres081 Project (recommended by This et al. 2004) available in the Italian *Vitis* database (www.vitisdb.it), the European *Vitis* database (www.eu-vitis.de) and the *Vitis* International Variety Catalogue (www.vivc.de) for identification of synonyms. The varieties used for SSR standardization were *Malvasia di Lipari* for the Italian and European databases, and *Sangiovese* for the International database. The SSR profile search engine of the Italian database allowed a tolerance of ±1 nucleotide, accounting for the scatter pattern. Conversely, the European and International databases only retrieved varieties with 100 % bp match. Cases of homonymy were identified checking for names in the same databases. Several diversity parameters were estimated using GenAlEx 6.5 (Peakall and Smouse 2012): the number of alleles per locus (N_a), the allele size range and the allele frequency, and the observed (H_o) and expected (H_e) heterozygosity (Nei 1978, 1987). Estimated frequency of null alleles (r) was calculated with the software IDENTITY (Wagner and Sefc 1999). Microsatellite screening ability (MSA) was also based on the probability of identity (PI) (Paetkau et al. 1995) and the polymorphic information content (PIC) (Weber 1990) derived as follows:

$$PI = \sum_{i=1}^{n} p_i^4 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

$$PIC = \left(\sum_{i=1}^{n} p_i^4 \right) - \left(\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2 \right)$$

where p_i and p_j are the frequencies of the ith and jth allele and n is the number of alleles. The PIC was directly calculated starting from H_e and PI. The above-mentioned indices range from 0.0 to 1.0 and provide information on the effectiveness to differentiate among genotypes. Thus, the most effective SSR has high values of H_o and polymorphic information content, and low PI.

The pairwise genetic distances among genotypes were calculated with the software Populations 1.2.31 (Langella 2002) using Nei's coefficient (Nei et al. 1983). Cluster analysis was performed according to UPGMA (Unweighted Pair-Group Method with Arithmetical Averages) algorithm and a dendrogram by using Populations 1.2.31 was generated and visualized with TreeGraph 2.0 (Stöver and Müller 2010).

Results and discussion

Distribution, use and peculiarities of the sampled grapevines

During our surveys we detected 93 different cultivars, as determined by farmers' claims (Table 2). Most of the surveyed cultivars were found just as relicts in old vineyards or nearby local wine cellars (ESM1 A-F). Consequently, many varieties are represented by a single sample. Other cultivars were more common throughout the territory and we collected sample material from several plants (e.g. up to 11, for *Zibibbo*). Out of the 93 cultivars, 39 were exclusive from Salina, 15 from Pantelleria, 23 from the Pelagie Islands, and 6 from Ustica. Some cultivars were present in two different archipelagos, i.e.: *Lacrime i Madonna was* found at Pantelleria and Ustica; *Zibibbo nero* at Salina and Pantelleria; *Inzolia* both at Salina and Pelagie; *Inzolia nera* and *Trummana* both at Salina and Ustica; *Funcia chiatta* and *Nave* both at Pantelleria and Pelagie. Only a few cultivars were present in three or all four archipelagos: *Catarratto* at Salina, Pantelleria and Pelagie; *Minna i vacca* at Pantelleria, Pelagie and Ustica; *Zibibbo* in all the archipelagos. The particular richness of cultivars at Salina confirms in this island the long agricultural and winery tradition, which is still persisting nowadays.

A number of cultivars already known from the literature as grown before the phylloxera outbreak still persist in some islands and must be regarded as "ancient" (Calcara 1853; Calcagno 1879; Habsburg Lothringen 1894). Besides the most renowned *Zibibbo*, *Catarrato* and *Inzolia*, it is especially the case of *Pignatello*, *Minna i vacca*, *Funcia chiatta*, *Nivuro* and *Greca* from Pantelleria, *Nuciddara*, *Mantuonica*, *Livedda* and *Trummana* from Salina, and *Alivedda nera* and *Lacrime i Madonna*, from Ustica.

Table 3 Genetic parameters at the 6 SSR loci analysed in the grapevine sampled cultivars

Locus	Na	Allele size range (bp)	H_e	H_o	r	PI	PIC	D_j
VVS2	10	127–149	0.827	0.867	−0.0218	0.0509	0.7850	0.8379
VVMD5	10	220–240	0.844	0.880	−0.0194	0.0427	0.8074	0.8557
VVMD7	11	231–261	0.769	0.773	−0.0027	0.0813	0.7130	0.7789
VVMD27	8	176–190	0.799	0.853	−0.0300	0.0686	0.7427	0.8101
VrZAG62	12	176–200	0.841	0.947	−0.0601	0.0440	0.8035	0.8521
VrZAG79	11	234–258	0.788	0.853	−0.0366	0.0690	0.7399	0.7985
Mean	10.33	–	0.811	0.862	–	0.0594	0.7653	0.8222
All Loci	62	–	–	–	–	3.672E−08	–	–

Na—number of Alleles per locus, H_e—expected heterozygosity, H_o—observed heterozygosity, r—estimated frequency of null alleles, PI—probability of identity, PIC—polymorphic information content, D_j—discrimination power

Table 2 summarizes the information on the use, putative origin and curiosities associated to the accessions, as retrieved by the interviews. Many varieties (40) were only used for wine making; 22 varieties were used for fresh table consumption; 19 for both wine making and table consumption. Only 12 varieties were used as dried fruit in addition to either wine making or fresh consumption, especially at Salina (*Corinto*, *Minnulettina*, *Minnilottina, Minutidda* and *Nuciddara*) and Pantelleria (the group of *Zibibbo* grapes).

For most of the varieties (61), the interviews revealed a number of distinctive peculiarities, often strictly bound to local customs (Table 2). For example, a couple of varieties (*Funcia chiatta* and *Nave*) were traditionally preserved in alcohol in the Pelagie islands, to be used by farmers as energizing snack in wintertime. The grapes of five varieties (*Cuda i vulpe*, *Malvasia* and *Nucignola/Rucignola* in Salina; *Nìvuro/Nivuro nostrale/Pignatello* and *Zibibbo* in Pantelleria) were sun-dried a few weeks before pressing in order to obtain sweeter and stronger wines. In particular, the musts from *Nìvuro* (meaning "black" in Sicilian language)/*Nivuro nostrale/Pignatello* are very dark so they were used to darken musts of other varieties. *Nìvuro/Nivuro nostrale/Pignatello* was appreciated over the centuries for its tannic structure and was much in vogue for the production of concentrated grape must (D'Agata 2014). The 'Ribollito' wine, produced by adding gypsum powder to *Nìvuro/Nivuro nostrale/Pignatello* grapes before pressing (Raja 1910), was a wine traditionally drunk in Pantelleria during Carnival. Nowadays, only *Malvasia* and *Zibibbo* are still used for making sweet wines, which play an important role in the economies of Salina and Pantelleria, respectively.

A quite interesting case concerned the cultivar *Citana* because of the unclear origin of its name, and the use of its grapes as well. According to two interviews, the name would derive from the Sicilian word "*acìtu*", meaning vinegar, indicating the unsuitability of the variety for making good wine. On the contrary, an old reference (Di Rovasenda 1877) mentions a juicy grapevine variety called *Acitàna* cultivated in the territory of Messina, whose name could mean it is native from the nearby village Acitrezza (district of Catania) whose inhabitants are called *acitàni* (Anon 1890).

Genetic identity and relevance of the investigated germplasm

The main genetic parameters related to the nuclear microsatellite diversity are reported in Table 3. The analyses revealed 62 alleles, ranging from 8 (VVMD27) to 12 (VrZAG62), with an average of 10.33 alleles per locus. The expected heterozygosity H_e (expressing gene diversity) ranged from 0.769 (VVMD7) to 0.844 (VVMD5), with a mean value 0.811, while the observed heterozygosity H_o ranged from 0.773 (VVMD7) to 0.947 (VrZAG62). For all loci, H_o was higher than H_e. The probability of null alleles was always negative and very close to 0 indicating the low probability of null alleles at all studied loci. When only one allele per locus was detected, samples were considered homozygous

Table 4 SSR standardized profiles of the grapevine sampled cultivars

	Local cultivar name and sampling area[a]	VVS2		VVMD5		VVMD7		VVMD27		VrZAG62		VrZAG79	
		1A	2A	1A	2A	1A	2A	1A	2A	1A	2A	1A	2A
1	Albanella bianca (US)	145	151	228	234	239	253	180	190	196	202	247	251
2	Albanello (US)	133	145	228	232	239	253	180	190	196	202	247	251
3	Alicante A (PA)	137	145	228	242	239	239	180	194	188	196	251	259
4	Alicante B (PA), Bertuccio A (PE), Catarratto rosato (PE), Nivuro B (PA)	143	145	228	230	239	239	182	186	186	188	251	259
5	Alivedda nera (US)	133	145	234	234	239	243	186	194	188	188	247	251
6	Aurora (US), Inzolia imperiale (AE)	133	135	228	234	239	249	186	186	186	188	243	251
7	Bertuccio B (PE), Racina i mustu D (AE)	143	151	228	242	239	249	180	182	186	202	251	251
8	Bertuccio C (PE), Calabrisi (PE), Gallipoli nera B (PE), Magliocco (AE), Mascarisi (AE), Nivureddu (PE), Nivureddu acino grosso (PE), Nivureddu antico (PE), Nivureddu arricciato (PE), Nivureddu grosso (PE), Nivureddu lasco (PE), Nivuro A (PA), Nivuro Nostrale/Pignatello (PA)	133	135	230	238	239	239	184	186	188	196	243	247
9	Caleu (PA)	133	151	242	242	233	243	184	194	188	204	247	257
10	Cantaro (AE)	133	143	236	248	233	249	180	180	188	204	247	247
11	Cappuccio (AE)	133	143	234	238	247	249	190	194	196	202	249	251
12	Catarratto (AE, PA), Catarratto acino grosso (AE), Catarratto acino piccolo (AE), Catarratto di Pantelleria (PA)	143	151	228	228	239	249	180	180	200	202	251	251
13	Catarratto bianco (PE), Nave C (PE), Squagghiammucca A (PE)	133	143	228	238	233	253	182	186	196	200	251	259
14	Centorotoli A (PA), Lacrime i Maria A (AE), Lacrime i Madonna (US)	137	149	228	242	233	239	186	194	188	204	251	257
15	Centorotoli B (AE)	133	143	228	234	249	253	180	184	194	200	245	251
16	Citana A (AE)	133	145	234	242	239	239	180	190	188	196	243	251
17	Citana B (AE), Putrisa (AE)	143	151	230	242	239	253	182	186	188	188	251	259
18	Corinto (AE), Minutidda (AE)	143	151	230	242	239	253	182	186	188	188	251	259
19	Cornicchiola (AE)	145	149	238	248	247	249	180	182	196	204	251	251
20	Cuda i vulpe (AE)	143	145	228	236	249	253	180	186	188	202	237	251
21	Damaschino (US), Paradiso B (PE), Spogliammucca (PE)	143	145	230	242	239	243	180	194	186	188	251	257
22	Diretta (AE)	133	143	234	238	237	251	182	190	180	194	255	259
23	Diretta bianca (AE)	133	135	238	240	249	251	186	190	186	194	239	261
24	Diretta nera (AE)	139	143	230	246	237	239	180	190	186	198	249	251
25	Fiore d'arancio (AE), Trunzu (AE)	135	151	236	238	247	253	180	194	196	204	251	257
26	Funcia chiatta (PE)	143	149	228	242	239	239	180	194	186	188	251	257
27	Gallipoli bianca A (PE)	133	143	228	238	233	239	182	186	188	196	247	251
28	Gallipoli bianca B (PE)	143	149	228	230	249	249	180	194	186	202	247	251
29	Gallipoli nera A (PE)	143	145	228	230	239	239	182	186	188	196	243	247
30	Garignano A (PA)	133	143	228	228	239	239	182	186	186	188	251	259
73	Garignano B (PA)	143	145	228	230	239	249	182	186	186	188	251	259
31	Giugnatica (AE), Vugliatico (AE)	133	143	230	238	239	247	186	190	194	204	251	259
32	Greca (PA)	137	143	240	242	233	239	186	186	188	204	251	257
33	Inzolia A (AE, PE), Nave D (PE)	135	143	228	242	239	247	180	184	188	202	247	251
34	Inzolia B (PE)	133	135	238	242	239	247	184	186	188	198	247	251

Table 4 continued

	Local cultivar name and sampling area[a]	VVS2		VVMD5		VVMD7		VVMD27		VrZAG62		VrZAG79	
		1A	2A	1A	2A	1A	2A	1A	2A	1A	2A	1A	2A
35	Inzolia Linosana (PE)	143	143	242	242	239	239	180	180	186	202	251	257
36	Inzolia nera A (AE)	133	137	240	242	239	249	180	194	186	202	251	257
37	Inzolia nera B (US)	133	133	234	238	253	257	180	194	194	200	251	251
38	Inzolia Tunisina A (PA)	135	143	238	242	243	249	182	190	188	204	243	247
39	Inzolia Tunisina B (PA)	145	149	238	248	239	247	182	182	188	204	247	251
40	Lacrime i Madonna (PA)	145	149	238	248	233	239	182	182	188	204	251	257
41	Lacrime i Maria B (AE)	149	153	228	234	239	239	180	194	188	204	247	257
42	Livedda (AE)	137	151	236	240	233	233	182	194	196	204	251	257
43	Lugliatica/Luglienga (AE)	143	143	228	228	243	249	180	184	188	202	243	247
44	Maddalena Salamone (PA)	135	135	238	240	243	247	182	182	188	204	259	259
45	Malvasia (AE)	143	145	228	228	243	249	180	184	188	202	243	247
46	Mantonico A (AE)	133	133	228	234	239	249	180	194	196	200	247	249
47	Mantonico B (AE), Pirricone raspo rosso (AE)	133	135	228	240	249	255	186	186	186	204	239	251
48	Maria Pirovano (PA)	145	149	230	236	239	249	194	194	186	188	247	259
49	Minna i Vacca A (PA), Minna i vacca Linosana (PE)	133	155	236	240	233	247	180	194	196	204	247	257
50	Minna i vacca B (US)	133	149	234	240	243	247	180	194	192	204	255	257
51	Minna i vacca C (PA)	137	149	234	240	239	243	180	186	188	188	247	257
52	Minna i vacca D (PE)	135	135	228	238	249	249	180	186	186	186	251	255
53	Minnilottina (AE), Minnulettina (AE)	137	139	230	240	233	239	186	194	186	204	251	257
54	Moscato nero (AE), Zibibbo nero A (AE)	135	149	234	240	247	249	180	186	186	192	239	255
55	Nave A (PA)	133	143	230	242	239	243	182	190	188	196	243	257
56	Nave B (PA)	133	143	228	240	239	243	182	190	188	196	243	251
57	Nera da vino (PE)	133	143	228	242	239	239	182	186	186	188	251	259
58	Nerello Mascarisi (AE)	135	135	230	238	239	239	184	186	188	196	243	247
59	Nero d'Avola (PE)	133	133	228	238	239	249	180	186	194	196	243	259
60	Nivureddu acino piccolo (PE)	133	133	228	238	239	249	180	186	194	196	243	259
61	Nuciddara (AE)	143	151	236	242	249	249	186	190	200	202	237	243
62	Paradiso A (PE), Squagghiammucca B (PE)	133	151	230	242	239	257	180	186	188	194	251	251
63	Perricone (AE)	133	155	242	242	239	247	184	186	188	198	247	251
64	Pizzitello bianco (PA), Pizzutella bianca (PE)	133	149	240	248	239	249	180	188	188	204	251	251
65	Racina i mustu A (AE)	143	143	236	242	239	249	182	194	188	204	251	251
66	Racina i mustu B (AE)	133	143	238	242	249	263	180	186	194	202	243	251
67	Racina i mustu C (AE)	133	145	228	230	247	253	180	180	196	200	249	255
68	Racina i ventu (AE)	133	143	238	242	239	249	180	186	202	204	247	251
69	Regina (PA)	133	155	238	238	249	249	180	182	186	204	255	259
70	Rucignola (AE)	143	145	228	242	249	249	186	192	200	202	243	247
71	Sfaghesina (PE)	143	155	236	242	239	247	180	194	186	196	247	257
72	Trummana (AE, US)	149	153	228	234	239	251	180	194	188	204	247	257
74	Zibibbo (PA), Zibibbo a grappolo spargolo (PA), Zibibbo Antico (PA), Zibibbo bianco (AE), Zibibbo dorato (PA), Zibibbo minna i vacca (PA), Zibibbo nero B (PA)	133	149	230	234	249	251	180	194	186	204	247	255

Table 4 continued

	Local cultivar name and sampling area[a]	VVS2		VVMD5		VVMD7		VVMD27		VrZAG62		VrZAG79	
		1A	2A	1A	2A	1A	2A	1A	2A	1A	2A	1A	2A
75	Zu Manuele (US)	137	151	228	234	239	249	180	192	186	202	251	251

The varieties used for SSR standardization were *Malvasia di Lipari* for the Italian and European databases, and *Sangiovese* for the International database

[a] Enclosed within brackets, is reported the sampling area: AE—Aeolian Islands (Salina), PA—Pantelleria Island, PE—Pelagie Islands (Lampedusa and Linosa), US—Ustica Island

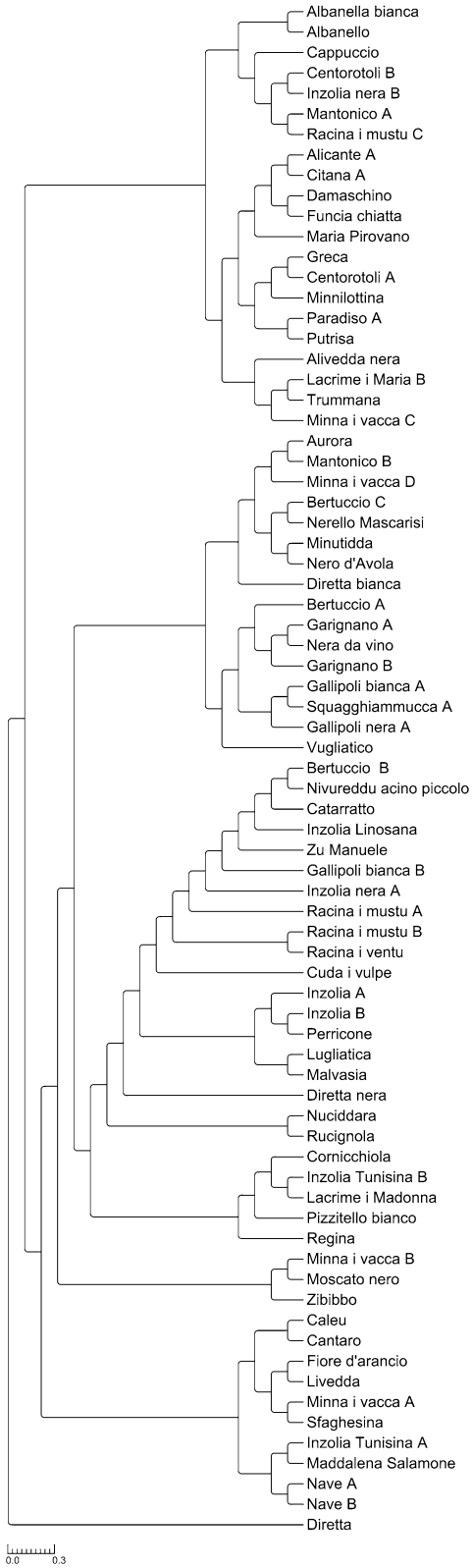
genotypes rather than heterozygous with a null allele. The most informative locus was VVMD5, with PI of 0.0427. The six SSR loci we used showed a high discrimination power (0.8222) and a low probability that two randomly chosen individuals had identical genotypes (PI 3.67E − 08). According to that, cultivars with identical profiles were considered synonyms.

The analyses at six SSR microsatellites revealed 75 different genotypes (Table 4; Fig. 2), fewer than the 93 different denominations given by farmers. Accordingly, in 20 cases each genotype was known by at least two synonyms (Table 4). In some cases, such as for *Cataratto* and *Zibibbo*, the same genotype was indicated even by several names mainly referred to distinctive traits of the berry (e.g. big, small, golden, and so on) in comparison to the standard type, so deserving a specific qualification and a new naming (Table 4). In other cases, the cultivar names were clearly different (Table 4) and most often restricted to the island of provenance. For example, the cultivar name *Nivureddu* and all its variants (*Bertuccio C*, *Calabrisi* and *Gallipoli nera B*) (Table 4, accession n. 8) was found in the Pelagie Islands, the synonym *Nìvuro nostrale/Pignatello* in Pantelleria, and *Magliocco* and *Mascarisi* in Salina. It can be assumed, therefore, that the diffusion of a cultivar in a new island was not always accompanied by its original denomination, then taking a local new name. In other cases, synonyms (e.g. *Bertuccio A/Catarratto rosato*, *Fiore d'arancio/Trunzu*) were found within the same island. It is noteworthy that in the case of *Fiore d'arancio/Trunzu*, despite farmers were fully aware of the coincident identity of these two cultivars, they used alternative denomination in the southern and northern parts of Salina, respectively. This occurrence could correlate with the mountainous topography of the island, which acted as a kind of barrier separating its territory in two (later on three) independent and even competing municipalities.

Another interesting case of synonymy concerns the cultivars *Giugnatica and Vugliatico*. These plants, provided by two different farmers of Salina, were reported to be named according to the different time of fruits ripening (*Giugnatica* from "Giugno" = June, *Vugliatico* from "Luglio" = July). Since genetic analysis revealed they were actually identical, their presumptive asynchronous maturation could therefore depend on the different environmental conditions of the growing sites. A third cultivar with a maturation-related name, *Lugliatico* or *Luglienga* (from "Luglio" = July), was instead a truly distinctive genotype (Table 4). It has to be noted that some cases of synonymy indicating phenological or morphological variants are most likely due to the influence of different environmental conditions.

The cases of homonymy were abundant as well, since in 18 cases one single cultivar name actually referred to different genotypes. Overall, this result proved the efficiency of the six selected SSRs to discern among plants with very similar phenotype. In Table 4 we discriminated among these profiles by attributing a suffix to the given name (e.g. *Alicante A*, *Alicante B*). Generally, cultivars with homonyms were considered of minor value by farmers, probably accounting for the limited attention toward possible phenotypic differences.

In a couple of striking cases, e.g. *Minna i vacca* and *Racina i mustu*, the same name was shared by four genotypes (Table 4, Fig. 2). Apparently, their peculiar phenotypic characteristics triggered the attribution of evocative denominations (in Sicilian language, *Minna i vacca* means "*Cowteat*" and refers to the elongate berry shape; *Racina i mustu* means "*Must grape*" and indicates the special suitability of the cultivar for wine making).



◀**Fig. 2** Dendrogram of genetic relationship among the investigated cultivars

The comparison of the 75 genetic profiles with national and international grapevine databases—namely the Italian *Vitis* Database, the European *Vitis* Database and the *Vitis* International Variety Catalogue—allowed assessing the extent of their diffusion and/or correspondence with more renowned cultivars. Only 36 genetic profiles corresponded to known varieties. The attributed area of origin as inferred by the databases was mainly Mediterranean, with most varieties from Italy (14), followed by France (5), Greece (3), Lebanon (2), Spain (2) and Tunisia (2). Croatia, UK, USA all accounted for one variety each. On the contrary the others profiles (39), were not included within any database and are therefore to be considered as new genotypes (Table 5). Phylogenetic analysis of the cultivars, based on the six SSR's, did not reveal any geographic pattern. Accessions collected in the same island are scattered in different clusters attesting no gene exchange between putative local parents and then their different origin and history of introduction (Fig. 2). This result was expected, given the central position of the circum-Sicilian islands within the trade routes of the Mediterranean Basin as well as their complex history of colonization. Three varieties (*Malvasia*, *Maria Pirovano* and *Zibibbo*), though listed in the International databases, are not referred to a specified country of origin herein. According to data from literature, the origin of *Malvasia*, initially thought to be Greek, remains actually obscure (Crespan et al. 2015). *Maria Pirovano* is a recent variety created in 1926 by the breeder Alberto Pirovano (cf. www.vivc.de), whereas *Zibibbo* is considered native from Tunisia (Niccoli 1902).

In some cases (e.g. *Catarratto*, *Damaschino*, *Malvasia* and *Zibibbo*) the appellation given by the farmers corresponded to the official name as recorded in the databases. All of them are important varieties for the current wine industry of Sicily and its minor islands, and therefore they are largely known and characterized since long time. Other varieties showed evident similarity to the official nomenclature (*Cornicchiola–Cornichon*, *Inzolia–Ansonica*, *Maddalena Salomone–Madeleine Angevine Oberlin and Moscato*

Table 5 Comparison of genetic profiles with international databases

Local names and sampling area[a]	Correspondent SSR profile in Italian *Vitis* database (www.vitisdb.it)	Correspondent prime name for SSR profile in European *Vitis* database (www.eu-vitis.de)	Correspondent prime name for SSR profile in *Vitis* International Variety Catalogue (www.vivc.de)	Cases of homonymy	Country of origin
Albanella bianca (US)	–	–	Guardavalle		Italy
Albanello (US)[b]	–	–	–	VIVIC	–
Alicante A (PA)[b]	–	–	–		–
Alicante B (PA), Bertuccio A (PE), Catarratto rosato (PE), Nivuro B (PA)	Nerello cappuccio	Carignan	Carignan noir		France
Alivedda nera (US)	–	–	Prune de Cazouls		–
Aurora (US), Inzolia imperiale (AE)	–	Afus Ali	Afus Ali	EU, VIVIC	Lebanon
Bertuccio B (PE), Racina i mustu D (AE)	Calabrese	Calabrese	Calabrese		Italy
Bertuccio C (PE), Calabrisi (PE), Gallipoli nera B (PE), Magliocco (AE), Mascarisi (AE), Nivureddu (PE), Nivureddu acino grosso (PE), Nivureddu antico (PE), Nivureddu arricciato (PE), Nivureddu grosso (PE), Nivureddu lasco (PE), Nivuro A (PA), Nivuro nostrale/ Pignatello (PA)	–	–	Perricone		Italy
Caleu (PA)[b]	–	–	–		–
Cantaro (AE)	–	Mijajusa	Asswad Karech, Mijajusa		Lebanon
Cappuccio (AE)[b]	–	–	–		–
Catarratto (PA, AE), Catarratto acino grosso (AE), Catarratto acino piccolo (AE), Catarratto di Pantelleria (PA)	–	Catarratto bianco comune	–		Italy
Catarratto bianco (PE), Nave C (PE), Squagghiammucca A (PE)[b]	–	–	–	VIVIC	–
Centorotoli A (PA), Lacrime i Maria A (AE), Lacrime i Madonna (US)	–	–	Lacrime di Maria[c]		Italy
Centorotoli B (AE)	Trebbiano toscano	Trebbiano toscano	Trebbiano toscano		Italy
Citana A (AE)	Pampanuto, Verdeca	–	Lagorthi		Greece
Citana B (AE), Putrisa (AE)[b]	–	–	–		–
Corinto (AE), Minutidda (AE)	Sangiovese	Sangiovese	Sangiovese	VIVIC	Italy
Cornicchiola (AE)	–	Dedo de dama	Cornichon blanc, Dedo de Dama		Italy
Cuda i vulpe (AE)	–	–	Frmentum	VIVIC	Croatia
Damaschino (US), Paradiso B (PE), Spogliammucca (PE)	Damaschino	–	Planta fina[c]		Italy

Table 5 continued

Local names and sampling area[a]	Correspondent SSR profile in Italian *Vitis* database (www.vitisdb.it)	Correspondent prime name for SSR profile in European *Vitis* database (www.eu-vitis.de)	Correspondent prime name for SSR profile in *Vitis* International Variety Catalogue (www.vivc.de)	Cases of homonymy	Country of origin
Diretta (AE)[b]	–	–	–		–
Diretta bianca (AE)	–	–	Dattier de St. Vallier		France
Diretta nera (AE)[b]	–	–	–		–
Fiore d'arancio (AE), Trunzu (AE)	–	–	Korithi Aspro	VIVIC	Greece
Funcia chiatta (PE)	–	–	Rassegui		Tunisia
Gallipoli bianca A (PE)[b]	–	–	–		–
Gallipoli bianca B (PE)	Grillo	–			Italy
Gallipoli nera A (PE)[b]	–	–	–		–
Garignano A (PA)[b]	–	–	–		–
Garignano B (PA)[b]	–	–	–		–
Giugnatica (AE), Vugliatico (AE)	–	Chasselas temprano blanco/colorado	Chasselas blanc, rosé, rouge		France
Greca (PA)[b]	–	–	–	VIVIC	–
Inzolia A (AE, PE), Nave D (PE)	–	Ansonica	Ansonica	EU, IT, VIVIC	Italy
Inzolia B (PE)[b]	–	–	–	EU, IT, VIVIC	–
Inzolia Linosana (PE)[b]	–	–	–		–
Inzolia nera A (AE)[b]	–	–	–		–
Inzolia nera B (US)	–	–	Aubun		France
Inzolia Tunisina A (PA)	–	Beba	Beba		Spain
Inzolia Tunisina B (PA)[b]	–	–	–		–
Lacrime i Madonna (PA)[b]	–	–	–		–
Lacrime i Maria B (AE)[b]	–	–	–	VIVIC	–
Livedda (AE)[b]	–	–	–		–
Lugliatica/Luglienga (AE)[b]	–	–	–	EU, VIVIC	–
Maddalena Salamone (PA)	–	Madeleine Angevine Oberlin	Madeleine Angevine Oberlin	VIVIC	France
Malvasia (AE)	Malvasia di Lipari	Malvasia di Lipari	Malvasia di Sardegna		–
Mantonico A (AE)[b]	–	–	–	IT, VIVIC	–
Mantonico B (AE), Pirricone raspo rosso (AE)	–	Alphonse La Vallée	Alphonse La Vallée	IT, VIVIC	France
Maria Pirovano (PA)	Signurina	–	Maria Pirovano[c]		–
Minna i vacca A (PA), Minna i vacca Linosana (PE)	–	–	Bezoul El Khadem de Tunisie		Tunisia
Minna i vacca B (US)	–	–	Italia[c]		Italy
Minna i vacca C (PA)[b]	–	–	–		–
Minna i vacca D (PE)	–	Cardinal	Cardinal		United States
Minnilottina (AE), Minnulettina (AE)[b]	–	–	–		–

Table 5 continued

Local names and sampling area[a]	Correspondent SSR profile in Italian *Vitis* database (www.vitisdb.it)	Correspondent prime name for SSR profile in European *Vitis* database (www.eu-vitis.de)	Correspondent prime name for SSR profile in *Vitis* International Variety Catalogue (www.vivc.de)	Cases of homonymy	Country of origin
Moscato nero (AE), Zibibbo nero A (AE)	–	Muscat Hamburg	Muscat Hamburg		United Kingdom
Nave A (PA)	–	Tortosina	Tortosina		Spain
Nave B (PA)[b]	–	–	–	EU, VIVIC	–
Nera da vino (PE)[b]	–	–	–		–
Nerello Mascarisi (AE)[b]	–	–	–	VIVIC	–
Nero d'Avola (PE)[b]	–	–	–		–
Nivureddu acino piccolo (PE)[b]	–	–	–		–
Nuciddara (AE)[b]	–	–	–		–
Paradiso A (PE), Squagghiammucca B (PE)[b]	–	–	–		–
Perricone (AE)	–	–	Catanese nero	VIVIC	Italy
Pizzitello bianco (PA), Pizzutella bianca (PE)[b]	–	–	–		–
Racina i mustu A (AE)[b]	–	–	–		–
Racina i mustu B (AE)[b]	–	–	–		–
Racina i mustu C (AE)[b]	–	–	–		–
Racina i ventu (AE)[b]	–	–	–		–
Regina (PA)[b]	–	–	–		–
Rucignola (AE)	–	–	Rucignola		Italy
Sfaghesina (PE)[b]	–	–	–		–
Trummana (AE, US)	–	–	Heptakilo[c]		Greece
Zibibbo (PA), Zibibbo a grappolo spargolo (PA), Zibibbo antico (PA), Zibibbo bianco (AE), Zibibbo dorato (PA), Zibibbo minna i vacca (PA), Zibibbo nero B (PA)	Zibibbo	Muscat d'Alexandrie[c]	Muscat of Alexandria[c]		–
Zu Manuele (US)[b]	–	–	–		–

IT—Italian *Vitis* database, EU—the European *Vitis* database, VIVC—the *Vitis* International Variety Catalogue database

[a] Enclosed within brackets, is reported the sampling area: AE—Aeolian Islands (Salina), PA—Pantelleria Island, PE—Pelagie Islands (Lampedusa and Linosa), US—Ustica Island. Country of origin data source VIVC database

[b] New SSR profiles

[c] A manual correction of one nucleotide alleles in the databases was necessary, since the recorded profile had a wrong odd score

Nero–Muscat Hamburg), likely resulting from the transposition of the approved name into the local vernacular. However, the majority of varieties (25 out of 36) with a known profile had a name that differed completely from the official one, representing a glaring example of synonymy. In some cases, the re-naming could be attributed to difficult pronunciation of foreign terms by peasants, but it is not rare that the islander names also differed from those commonly used in other Italian regions or even in mainland Sicily. In this regard, striking examples were *Calabrese*, *Trebbiano toscano*, *Grillo* and *Sangiovese*, amongst the most famous varieties for the wine industry, that in the Sicilian minor islands have

alternative denominations (*Bertuccio B/Racina i mustu D*, *Centorotoli B*, *Gallipoli bianca B* and *Corinto/Minutidda,* respectively). Moreover, the comparison with the international databases highlighted several cases of homonymy (Table 5): e.g. the *Nero d'Avola* from the Pelagie archipelago is a case of homonymy, since it was genetically different from the official *Nero d'Avola* (De Lorenzis et al. 2014). Though the individual reasons for the different denominations remain obscure, we can hypothesise that those are cases of mislabelling that perpetuated in time. Additionally, the true name associated to a specific cultivar could have been lost during the successive generation of growers, and a new one was created/attributed later on. The geographical and cultural isolation of most of the circum-Sicilian islands, together with the very few farmers still active nowadays, make this type of "cultural mutation" very likely and persistent.

An interesting case of homonymy concerns the variety *Cuda i vulpe* found in Salina, that proved different from the official cultivar *Coda di Volpe* from the Campania region (Italy) (Costantini et al. 2005). It is worth mentioning that Salina, and the Aeolian archipelago in general, has been colonized by immigrants from Campania during the Borbonic Kingdom (eighteenth–nineteenth century). It is thus intriguing to imagine that the memory of a favourite grapevine variety survived the plants themselves, and was finally attributed to grapes with similar phenotype. The Aeolian *Cuda i vulpe* actually proved a synonym of *Frmentum*, a variety native to Croatia (Table 5). The explanation could rely on migratory fluxes that since 1561 occurred from the Venetian colonies in the Adriatic and eastern Mediterranean towards the Aeolian Archipelago, during which immigrants brought together a number of grapevine varieties (Archivio Storico Eoliano). Consistently, one farmer reported that the *Cuda i vulpe* variety was in fact native from Crete (Greece), the ancient Candia of the Venetian Republic (thirteenth–seventeenth century) (Table 2).

Interestingly, black *Mantonico* grapes are mentioned in a number of notarial deeds of sale in Syracuse since 1478 (Amato 1996) and, successively, 1555 in Ficarazzi (district of Palermo) (Morreale 1998), possibly meaning that the red grape *Mantonico A* from Salina, showing a new SSR profile, could be the same ancient variety.

Rare germplasm preservation resulted difficult due to the low quality (e.g. plants in abandoned fields, sanitary status, old vineyard) of the plant material available for plant propagation. The percentage of grapevine accessions survived in the ex situ collection field was low (60 %) and some accessions were lost. The ex situ collection field will allow us the ampelographic verification of the survived accessions in homogeneous growing conditions.

Conclusions

This work complements previous genetic characterization of varieties from mainland Sicily (Carimi et al. 2010, 2011; De Lorenzis et al. 2014). While we found commonalities with Carimi et al. (2010) (*Catarratto*, *Diretta bianca*, *Inzolia*, *Lacrime i Maria*, *Rucignola* and *Zibibbo*), but none with De Lorenzis et al. (2014), the vast majority (69 out of 75) of the varieties found in the circum-Sicilian minor islands were not reported until now in mainland Sicily. This study confirms that the minor islands of the Mediterranean Basin still represent underexplored hotspots of genetic diversity for grapevine. These important reservoirs of disregarded but potentially valuable genotypes could be of great value to breeders and the wine industry and they shed light on the migration of cultivars. In the small circum-Sicilian islands, the long-term survival of these rare varieties is uncertain, due to the increasing land abandonment and the shift of the economic drive from agriculture to tourism, a trend already observed in other islands (Pignone et al. 2001). During a field survey conducted 5 years later the first sample campaign, we found that 8 % of the sampled accessions had not survived. The urgent need to preserve this unique germplasm is testified by our own observations. Survey campaigns and collection fields become then an essential tool to preserve the grapevine germplasm and to avoid an irreversible loss of genetic diversity.

Acknowledgments This research was partly supported by Regione Siciliana (PSR Sicilia 2007–2013, Sottomisura 214/2A—Preservazione della biodiversità: Centri pubblici di conservazione, Grant No. 94750767637) and by the grant from the Italian Ministry of the Research in the frame of the project 'Tracciabilità della filiera vitivinicola' (OR 2.1.2-CISIA DGLS 191/2009). We are very grateful to all the following informants for providing material, interviews and information. Without

their support, openness, memory, passion for traditions and history of their peoples and territory, this work could not have been realized. They are the last holders of an unwritten tradition that is being lost. Together with them we have spent so many pleasant years of research. The farmers Pino Bartolo, Antonino Bono, Salvatore Maltese, Giuseppe Palmeri, Gaetano Taranto Pasquale Tonnicchia and, the personnel of Legambiente Association (Manager of Isola di Lampedusa Nature Reserve): Vincenzo Billeci, Giuseppe Maraventano, Giusi Nicolini, Elena Prazzi, Gerry Sorrentino from *Lampedusa*; Fedele Giardina, Salvatore La Russa and Giacomo Sorrentino from *Linosa*; Battista Belvisi, Giovanni Belvisi, Francesco Belvisi, Giovanni "Migroscio" Bonomo, Giovanni Consolo, Giovanni Errera, Antonio Ferrandes, Giovanni Ferrandes, Salvatore Ferrandes, Vito Ferreri, Michele Maccotta, Giovanni Maddalena, Giovanni Rizzo and Gabriele Vincenzo from *Pantelleria*; the farmers Lorenzo Basile, Gaetano Biscotto, Felicino Bruno, Antonio Cento, Salvatore Cincotta, Salvatore D'Amico, Carmela "Pizzicata" De Corrado, Lucio De Fina, Antonio Di Giorgi, Aldo Galletta, Salvatore Galletta, Rosario Giordano, Felice La Rosa, Gaetano Marchetta, Mario Marsile, Giacomo Merlino, Fiorella Miglietti, Antonino Mirenda, Mimì Randazzo, Bartolino Russo, Enrico Virgona and Gino Zagami and Pietro Lo Cascio and Flavia Grita (NESOS Association) from *Salina*; Nicola Longo and Margherita Longo from *Ustica*. In special remembrance of Carmelina De Corrado, who passed away last year at the age of 100.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Anon. (1890) Atti e rendiconti dell'Accademia di scienze, lettere e arti dei Zelanti e pp. dello studio di Acireale. Micale, Acireale

Abelli L, Agosto MV, Casalbore D, Romagnoli C, Bosman A, Antonioli F, Pierdomenico M, Sposato A, Latino Chiocci F (2014) Marine geological and archaeological evidence of a possible pre-Neolithic site in Pantelleria Island, Central Mediterranean Sea. Geological Society, London, Special Publications 411:SP411-6

Archivio Storico Eoliano. http://www.archiviostoricoeoliano.it/. Accessed 28 Oct 2015

Amato L (1996) Economia e Società nel quattrocento siracusano. Archivio Storico Siracusano 10(11):63–86

Bonasera F (1965) L'Isola di Pantelleria. Patron Editore, Bologna

Bowers JE, Dangl GS, Vignani R, Meredith CP (1996) Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). Genome 39:628–633

Calcagno M (1879) Dell'agricoltura dell'isola di Lampedusa. L'Agricoltore Calabro-Siculo 13:155–157

Calcara P (1853) Breve cenno sulla geognosia ed agricoltura dell'isola di Pantellaria. II parte. Giornale della Commissione per l'Agricoltura e la Pastorizia della Sicilia 2(4):270–281

Carimi F, Mercati F, Abbate L, Sunseri F (2010) Microsatellite analyses for evaluation of genetic diversity among Sicilian grapevine cultivars. Genet Resour Crop Evol 57:703–719

Carimi F, Mercati F, De Michele R, Fiore MC, Riccardi P, Sunseri F (2011) Intra-varietal genetic diversity of the grapevine (*Vitis vinifera* L.) cultivar 'Nero d'Avola' as revealed by microsatellite markers. Genet Resour Crop Evol 58:967–975

Civetta L, Cornette Y, Crisci G, Gillot PY, Orsi G, Requejos CS (1984) Geology, geochronology and chemical evolution of the island of Pantelleria. Geol Mag 121:541–668

Corti C, Lo Cascio P, Masseti M, Pasta S (2002) Storia naturale delle Isole Pelagie. L'Epos, Palermo

Costantini L, Monaco A, Vouillamoz JF, Forlani M, Grando MS (2005) Genetic relationships among local *Vitis vinifera* cultivars from Campania (Italy). Vitis 44:25–34

Crespan M, Cabello F, Giannetto S, Ibanez J, Kontić JK, Maletić E, Pejić I, Rodríguez-Torres I, Antonacci D (2015) Malvasia delle Lipari, Malvasia di Sardegna, Greco di Gerace, Malvasia de Sitges and Malvasia dubrovačka–synonyms of an old and famous grape cultivar. Vitis 45:69–73

D'Agata I (2014) Native wine grapes of Italy. University of California Press, California

D'Aietti A (1978) Il libro dell'isola di Pantelleria. Trevi Editore, Roma

De Gregorio R (1840) Descrizione delle principali isole adjacenti alla Sicilia. In: Capozzo Guglielmo, Memorie su la Sicilia tratte dalle più celebri accademie e da distinti libri di società letterarie e di valent'uomini nazionali e stranieri, con aggiunte e note per Guglielmo Capozzo vol 1. Virzì, Palermo, pp 171–182

De Lorenzis G, Las Casas G, Brancadoro L, Scienza A (2014) Genotyping of Sicilian grapevine germplasm resources (*Vitis vinifera* L.) and their relationships with Sangiovese. Sci Hortic 169:189–198

De Vita S, Laurenzi MA, Orsi G, Voltaggio M (1998) Application of Ar-40/Ar-39 and Th-230 dating methods to the chronostratigraphy of Quaternary basaltic volcanic areas: the Ustica Island case history. Quat Int 47:117–127

Di Bella M, Russo S, Petrelli M, Peccerillo A (2008) Origin and evolution of the Pleistocene magmatism of Linosa Island (Sicily Channel, Italy). Eur J Mineral 20:587–601

Di Lorenzo R, Carimi F, La Mantia T (2010) The Viticulture of Lampedusa, a heritage which is disappearing before being known. In: Proceedings 3rd international congress of mountain viticulture, Castiglione di Sicilia, 12–14 May 2010, pp 59–64

Di Rovasenda G (1877) Saggio di una ampelografia universale. Loescher, Torino, p 206

Doyle JJ, Doyle LJ (1987) A rapid DNA isolation procedure for small quantities of fresh leaf material. Phytochem Bull 19:11–15

Fernie AR, Tadmor Y, Zamir D (2006) Natural genetic variation for improving crop quality. Curr Opin Plant Biol 9:196–202

Garfì G, Mercati F, Fontana I, Collesano G, Pasta S, Vendramin GG, De Michele R, Carimi F (2013) Habitat features and genetic integrity of wild grapevine *Vitis vinifera* L. subsp. *sylvestris* (CC Gmel.) Hegi populations: a case study from Sicily. Flora 208:538–548

Gigante C (1968) Cossura. Monografa storica, filologica e geografica dell'Isola di Pantelleria. Glossario e toponomastica. Milano

Grasso M, Pedley HM (1988) The Pelagian Islands: a new geological interpretation from sedimentological and tectonic studies and its bearing on the evolution of the central Mediterranean area (Pelagian Block). Geol Romana 24:13–34

Habsburg Lothringen LS (1894) Die Liparischen Inseln. Achtes Heft: Allgemeiner Theil. Mercy, Praha

Habsburg Lothringen LS (1898) Ustica. Mercy, Praha

Hammer K, Laghetti G (2006) Small agricultural islands and plant genetic resources. IGV-CNR, Bari

Holloway RR, Lukesh SS (2001) Ustica II: the results of the excavations of the Regione Siciliana, Soprintendenza ai Beni Culturali ed Ambientali Provincia di Palermo in collaboration with Brown University in 1994 and 1999. Providence, R.I, Center for Old World Archaeology and Art, Brown University

King R, Young S (1979) The Aeolian Islands: birth and death of a human landscape. Erkunde 33:193–204

La Mantia T, Carimi F, Di Lorenzo R, Pasta S (2011) The agricultural heritage of Lampedusa (Pelagie Archipelago, South Italy) and its key role for cultivar and wildlife conservation. Ital J Agron 6e17:106–110

Langella O (2002) Populations, a free population genetic software. Version 1.2. 31. http://http://bioinformatics.org/populations/

Lo Cascio P, La Mantia T (2013) Le isole Eolie. In: ISPRA (ed) "Frutti dimenticati e biodiversità recuperata. Il germoplasma frutticolo e viticolo delle agricolture tradizionali italiane. Casi studio: Isole della Sicilia, Lombardia", Quaderni Natura e Biodiversità, vol. 5, pp 32–35

Lucchi F, Peccerillo A, Keller J, Tranne CA, Rossi PL (2013) The Aeolian Islands volcanoes. Geological Society memory, vol 37. The Geological Society, London

Morreale A (1998) La vite e il leone. Storia della Bagaria. Roma-Palermo, Ciranna

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Nei M, Tajima F, Tateno Y (1983) Accuracy of estimated phylogenetic trees from molecular data. J Mol Evol 19:153–170

Niccoli V (1902) Saggio storico e bibliografico dell'Agricoltura Italiana dalle origini al 1900. UTE, Torino

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. Mol Ecol 4:347–354

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539

Pignone D, Gladis T, Hammer K (2001) The influence of changing socio-economic factors on crop germplasm conservation—a case study. Schriften Genet Ressour 16:70–78

Puviani L (1916) L'agricoltura nell'isola di Pantelleria. Il Coltivatore 62:337–340

Radi G (1973) Tracce di un insediamento neolitico sull'isola di Lampedusa. Atti Soc Tosc Sci Nat Mem Ser B 79:197–205

Raja V (1910) Il vino ribollito. Tipografia di Monaco e Mollica, Catania

Rühl J, Pasta S (2007) Plant succession on Sicilian terraces. Ann Bot 7:111–126

Rühl J, Pasta S, La Mantia T (2005) Metodologia per lo studio delle successioni secondarie in ex coltivi: il caso studio di Pantelleria (Canale di Sicilia). Forest@ 2:388-398

Scarponi F (1939) Aspetti del problema viti-vinicolo di Pantelleria. Nuovi Annali dell'Agricoltura 19(20):294–332

Sefc KM, Regner F, Turetschek E, Glössl J, Steinkellner H (1999) Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. Genome 42:367–373

Stöver BC, Müller KF (2010) TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinform 11:7

Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

This P, Jung A, Boccacci P, Borrego J, Botta R, Costantini L, Crespan M, Dangl GS, Eisenheld C, Ferreira-Monteiro F, Grando S, Ibáñez J, Lacombe T, Laucou V, Magalhães R, Meredith CP, Milani N, Peterlunger E, Regner F, Zulini L, Maul E (2004) Development of a standard set of microsatellite references alleles for identification of grape cultivars. Theor Appl Genet 109:1048–1058

Thomas MR, Scott NS (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). Theor Appl Genet 86:985–990

Unwin T (2005) Wine and the vine: an historical geography of viticulture and the wine trade. Routledge, London

Wagner HW, Sefc KM (1999) IDENTITY 1.0. Centre for Applied Genetics, University of Agricultural Sciences, Vienna, 500

Weber JL (1990) Informativeness of human (dC-dA)n-(dGdT) n polymorphisms. Genomics 7:524–530