

# Earliest evidence of a primitive cultivar of *Vitis vinifera* L. during the Bronze Age in Sardinia (Italy)

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**Abstract** The discovery of the Nuragic culture settlement of Sa Osa, Cabras-Oristano, Sardinia, has made it possible to investigate the domestication status of waterlogged un-charred grape pips that were recovered from three wells dating from the Middle and Late Bronze Age (ca. 1350–1150 BC). Applying the stepwise linear discriminant analysis method, a morphological comparison of archaeological seeds and modern wild and cultivated Sardinian grapes pips was performed to determine the similarities between them. The results showed that the archaeological seeds from the Middle Bronze Age have intermediate morphological traits between modern wild and cultivated grape pips from Sardinia. In contrast, the analyses performed on the archaeological seeds from the Late Bronze Age showed a high degree of similarity with the modern cultivars in Sardinia. These results provide the first evidence of primitive cultivated *Vitis vinifera* in Sardinia

during the Late Bronze Age (1286–1115 cal BC, 2σ). This evidence may support the hypothesis that Sardinia could have been a secondary domestication centre of the grapevine, due to the presence of ancient cultivars that still exhibit the phenotypic characteristics of wild grapes.

**Keywords** Archaeological seeds · Grape pips · Secondary domestication · Seed morphology · Image analysis · Mediterranean Basin

## Introduction

*Vitis vinifera* L. ssp. *vinifera* (grapevine) is one of the most ancient and precious fruit-bearing plants in the world and it has played a role of primary importance for complex societies around the Mediterranean (Mangafa and Kotsakis 1996; Manen et al. 2003; Zohary et al. 2012). In 2010, FAOSTAT estimated that grapes are the most widespread crop. Approximately eight million hectares are cultivated, with 71 % being used for wine production, and the remainder being destined for other food purposes (Food and Agriculture Organization 2007).

Archaeological evidence of grape begins with traces of tartaric acid which have been detected in Neolithic pottery vessels at Hajji Firuz Tepe (5400–5000 BC) in the northern Zagros mountains of Iran (McGovern et al. 1996, 1997; Zohary et al. 2012). Other evidence of grape juice has been discovered at the Neolithic site of Dikili Tash in Greece during the 5th millennium BC (Valamoti et al. 2007; Pagnoux et al. 2014). Similar references to winemaking and early viticulture have been recorded in ancient Egypt in Old Kingdom tombs (ca. 2700 BC) in the eastern Mediterranean (McGovern et al. 1997). However, the presence of traces of wine is not considered an indicator of grapevine

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cultivation because wild grapes may also have been used in the production of fermented juice (McGovern 2003; Miller 2008).

The first evidence of grape domestication was found in the Levant from the Chalcolithic period site at Tell Esh-Shuna North in the Jordan valley (ca. 3700–3200 BC) and in the Early Bronze Age (EBA) at Jericho (ca. 3200–1900 BC) (Zohary 1995; Zohary et al. 2012). Archaeobotanical data on cultivated grape around the Mediterranean support its appearance during the first half of the 3rd millennium BC in southern Greece and Cyprus and during the 2nd millennium BC in the southern Balkans (Kroll 1991). Other archaeobotanical records have provided information on the gathering of grapes from wild vines and their use as food during the early Neolithic in European contexts (Zohary et al. 2012).

In Italy, archaeobotanical data from the Middle Bronze Age (MBA) has confirmed the presence of grape pips and pollen at San Lorenzo a Greve, Toscana (Bellini et al. 2008; Mariotti Lippi et al. 2009), however, the origins of viticulture during the BA are still being debated (Stika and Heiss 2013). The increase in archaeobotanical records of grape pips from the Iron Age onwards suggest that grape cultivation began during this period (Marvelli et al. 2013). However, it is not yet clear whether these finds represent the transmission of knowledge and techniques of viticulture by the Phoenicians and Aegeans or if they were from domestication by the local people (Marvelli et al. 2013). The data available for Spain suggest a direct relationship between the introduction of viticulture and the first contact with Phoenicians and Greek colonists from Phocaea (Gómez-Bellard et al. 1993; Buxó 1997, 2008; Buxó and Piqué 2008). In southern France, written sources and archaeobotanical remains show the beginnings of grape cultivation with the foundation of the Greek city of Massalia around 600 BC (Brun 2011; Bouby et al. 2013). Later, with the Roman colonisation, viticulture slowly spread inland from the coastal areas (Marinval 1997; Bouby and Marinval 2001; Figueiral et al. 2010; Bouby et al. 2013).

Today, it is widely accepted that the modern cultivars of *V. vinifera* are the result of the domestication of *V. vinifera* L. ssp. *sylvestris* (wild grape) (Olmo 1995; Cunha et al. 2007). Wild grape is a heliophilous liana that grows in riparian woodlands. Currently, its range of distribution extends from the Atlantic coast to the western Himalayas, and it is thought that this range has not significantly changed (Levadoux 1956; Arnold et al. 1998, 2005; Zohary et al. 2012). The main feature used to distinguish between wild and cultivated *Vitis* is the reproductive system: the wild grape is dioecious, whereas the cultivated grapevine has hermaphroditic flowers; however, it is possible to find a small percentage of hermaphroditism also in the wild grape populations (Zecca et al. 2010). Hermaphroditism is an

important attribute of cultivated grape, which is due to a genetic modification caused by the domestication process (Zecca et al. 2010; Zohary et al. 2012). The domestication process performed by early viticulturists most probably used vegetative propagation of clones by growing cuttings, which induced a significant change in the reproductive biology of the grape to self-pollination, that ensured increased fruit production of larger berries with higher sugar content (McGovern 2003; Zohary et al. 2012; Bacilieri et al. 2013).

However, the location and date of the origin(s) of the domesticated grape in the Mediterranean area are still under scientific debate (Stika and Heiss 2013). Several authors argue that, based on genetic tests that were performed on cultivated and wild grape plants, the origin may have occurred at independent secondary domestication sites in the western Mediterranean area, which is favoured by the widespread presence there of wild grape (Rivera-Núñez and Walker 1989; Sefc et al. 2003; Grassi et al. 2003, 2008; Arroyo-García et al. 2006). Genetic studies conducted by Arroyo-García et al. (2006) and De Mattia et al. (2008) suggested that Spain, Italy and France may be possible centres of secondary domestication because a high level of genetic diversity was observed among domesticated plants in western Europe and the Near East, which is attributed to the contribution of the *V. sylvestris* germplasm from western Europe. A slighter lower haplotype diversity of *V. vinifera* is observed in western Europe compared with the Near East. The genetic evidence suggests that *V. vinifera* cultivars from western Europe experienced introgression from local wild grape populations (Myles et al. 2011). Grassi et al. (2003) have suggested that Sardinia could be a valid location of secondary domestication because of its isolation and the presence of numerous wild grape populations and ancient cultivars that still display the phenotypic characteristics of wild grapes. Genetic analyses have highlighted the genetic patrimony of two autochthonous cultivars (Bovale Muristellu and Bovale Murru) which share 50 % of their alleles with wild grapes that are present in the same area (Grassi et al. 2003). This result is the first evidence of the possible presence of a secondary centre of grapevine domestication in Sardinia.

In the last 10 years, numerous studies have been performed to identify and group diaspores of *Vitis* according to the morphometric features of the grape pips, which are measured by applying image analysis techniques, and these results are statistically evaluated using a stepwise linear discriminant analysis (LDA). This approach is a valuable tool that allows discrimination between seeds of wild and cultivated plants (Kilic et al. 2007; Venora et al. 2007, 2009a, b; Bacchetta et al. 2011a; Grillo et al. 2011; Orrù et al. 2012, 2013). Currently, this method is a valid and non-destructive tool that is used to verify the state of grape

pip domestication in archaeobotanical studies (Gong et al. 2010; Terral et al. 2010; Bouby et al. 2013; Orrù et al. 2013; Pagnoux et al. 2014). All of these studies prove that the morphological traits of seeds, such as the shape, size and external ornamentations, are very important diagnostic traits in plant taxonomy studies.

The rescue excavation performed at Sa Osa (Cabras, Oristano) allowed us to investigate the domestication status of grape pips recovered from three wells dating from between the MBA and LBA (ca. 1350–1150 BC). The pips were preserved waterlogged and uncharred; this allowed a minimal amount of discrimination errors because distortions, which appear on charred seeds, were not observed (Smith and Jones 1990). In fact, waterlogged seeds are good samples that can be used to directly compare archaeological seeds with the modern examples in the reference collection (Bouby et al. 2013).

The primary goal of this work is to investigate the domestication status of grape pips found in the Sa Osa settlement and to verify the possibility that primitive cultivars of *V. vinifera* might have existed during the Bronze Age in Sardinia. This investigation is undertaken by evaluating the relationship of archaeological seeds dating from the MBA and LBA with cultivars and wild populations, using LDA.

## Archaeological background

Sardinia, with an area of 24,089 km<sup>2</sup>, is the second largest island in the Mediterranean. The strategic position of the island in the middle of the western Mediterranean basin has facilitated contact and trade with eastern and western civilisations (Usai and Lo Schiavo 2009).

The Nuragic culture has its foundations in the MBA, which is when communities started to mark their territorial dominance with over 8,000 *Nuraghes*, cyclopean towers. These massive buildings were elements of territorial control and had multiple roles, which included being fortified dwellings, animal shelters and food storage areas (Ugas 2006; Lilliu 2011).

During the Middle, Late and Final BA (ca. 1600–930 BC), the Nuragic societies developed commercial relationships with the Mycenaean and Minoan world and then with the island of Cyprus, as shown by an abundance of ox-hide-shaped copper ingots and metal working tools (Begemann et al. 2001; Usai and Lo Schiavo

2009). During the Iron Age (ca. 930–600 BC), the island was in the Phoenician commercial network and it was later conquered by the Carthaginians, who founded several colonies along the coast. In 238 BC, the island became part of the Roman Empire (Barreca 1986).

Archaeobotanical data shows an advanced development of agriculture since the EBA (Ucchesu et al. 2014). Cultivated plants are based on four main cereals, *Hordeum vulgare* L. var. *nudum* (naked barley), *H. vulgare* L. (hulled barley), *Triticum aestivum* L. (bread wheat) and *T. durum* L. (durum wheat) which are associated with three legumes, *Vicia faba* L. (broad bean), *Lens culinaris* Medik. (lentil) and *Pisum sativum* L. (pea) (Ucchesu et al. 2014).

Carbonised grape pips have been found from the EBA (2017–1751 cal BC, 2σ, Table 1) site at Monte Meana, Santadi—CI (Tanda et al. 2012), the LBA and early Iron Age site at Duos Nuraghes, Borore—NU (1300–900 BC) (Webster and Webster 1998; Bakels 2002) and the Iron Age (900–500 BC) at Genna Maria (Villanovaforru-Cagliari) (Badas 1987). However, their specific domestication status remains unknown (Bakels 2002; Ucchesu et al. 2014).

## The nuragic settlements of Sa Osa

The archaeological site of Sa Osa (39°54′51″N 8°32′32″E, 6 m a.s.l.) is located in the gulf of Oristano, in west-central Sardinia, 2 km from the current coast line. The site is situated on the alluvial plain of the river Tirso and is bordered by two ponds, Cabras to the north and Santa Giusta to the south (Fig. 1). A recent archaeobotanical study of the site of Sa Osa, dating between the MBA and the early Iron Age (1600–700 BC), recovered a large quantity of grape pips associated with the remains of *Ficus carica* L. (fig) (Ucchesu et al. 2014; Usai et al. 2012).

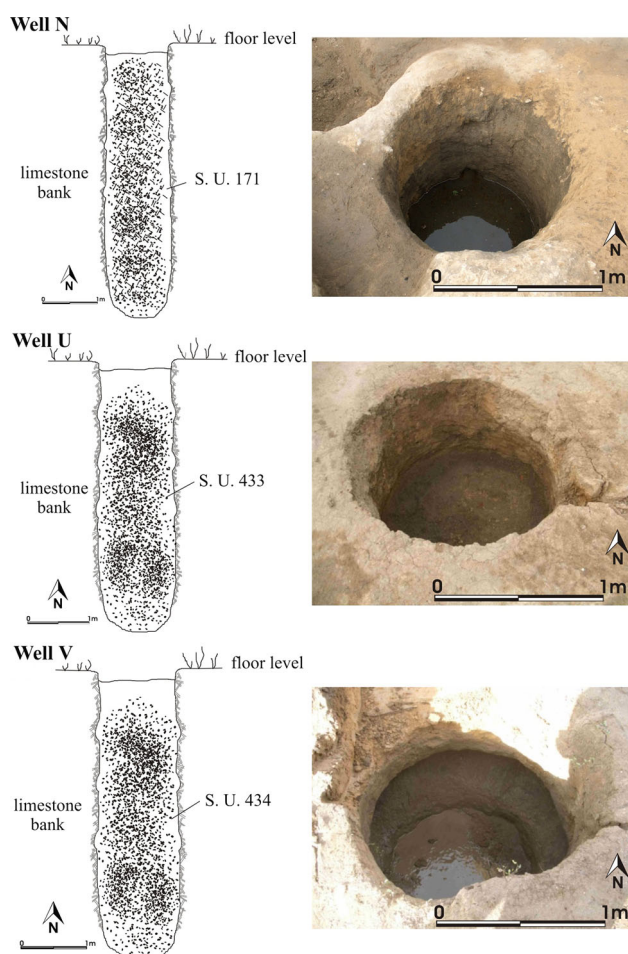
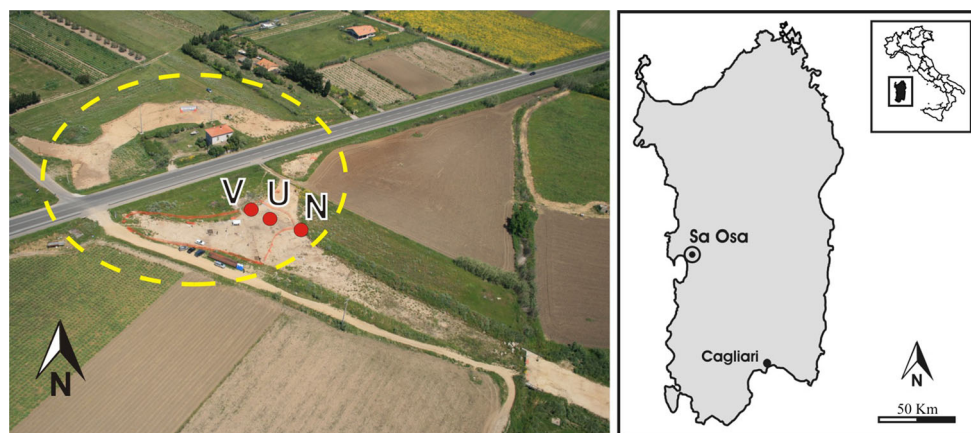
The three excavation seasons conducted between 2008 and 2009 by the Soprintendenza Archeologica di Cagliari and Università di Sassari confirmed the presence of several deep wells dug into the underlying sandstone, where a large quantity of plant remains of human origin were found. Several shallow pits and wells lacking any stone structures were observed, which represented living spaces from a time period between the MBA and the Iron Age (Usai et al. 2012).

In this study, the wells that yielded the largest quantity of *V. vinifera* remains, Wells N, U and V, were examined.

**Table 1** AMS dates of grape pips from Sa Osa calibrated with OxCal v4.2.3 (Bronk Ramsey and Lee 2013), r5, and the IntCal13 atmospheric curve (Reimer et al. 2013)

| Context           | Archaeological phase    | Lab. code   | Age (yrs BP) | Age (cal BC, 2σ) |
|-------------------|-------------------------|-------------|--------------|------------------|
| Well N (S.U. 171) | Late Bronze Age (LBA)   | OxA-25106   | 2,981 ± 27   | 1286–1115        |
| Well N (S.U. 171) | Late Bronze Age         | OxA-25107   | 2,968 ± 27   | 1276–1088        |
| Well U (S.U. 433) | Middle Bronze Age (MBA) | Beta-369365 | 3,000 ± 30   | 1377–1126        |
| Well V (S.U. 434) | Middle Bronze Age       | Beta-369366 | 3,020 ± 30   | 1391–1131        |

**Fig. 1** Location of the Sa Osa settlement and positions of Wells N, U and V



**Fig. 2** Sections of Wells N, U and V

The wells have circular mouths approximately 1.2 m wide and are 4–5 m deep. All of the layers consisted of damp sediment (Fig. 2). Well N was investigated to a depth of 5 m, and a large amount of animal bones, pottery and plant remains were found in it. Wells U and V were investigated to a depth of only 4 m because of safety reasons, and large

amounts of pottery, plant remains and animal bones were found there (Fig. 2; Usai et al. 2012).

## Materials and methods

### Archaeobotanical remains

A total of 15,000 waterlogged uncharred grape pips were retrieved from the Sa Osa settlement. Archaeological seeds from Well N were recovered from the stratigraphic unit (S.U.) 171 (Figs. 2, 3), whereas those from Wells U and V were recovered from S.U. 433 and S.U. 434, respectively (Figs. 2, 3).

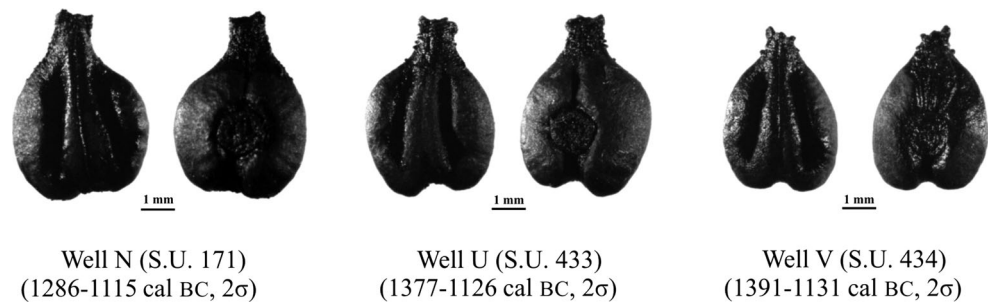
The archaeobotanical remains were extracted from the sediment with the wash-over technique using a fine mesh to collect them (0.25 mm) (Kenward et al. 1980). The seeds from each of these wells were subjected to four radiometric datings. The  $^{14}\text{C}$  dates acquired from two samples of grape pips were able to date Well N to the LBA (1286–1115 and 1276–1088 cal BC,  $2\sigma$ , Table 1). The radiocarbon dates acquired from grape pips found in Wells U (1377–1126 cal BC,  $2\sigma$ ) and V (1391–1131 cal BC,  $2\sigma$ ) both dated from the end of the MBA (Table 1). The clean material was kept in distilled water and stored at 5 °C in the Germplasm Bank of Sardinia (BG-SAR) (Atzeri et al. 2012) in the Centre for Conservation of Biodiversity at the University of Cagliari (Bacchetta et al. 2004, 2008a). A total of 2,009 archaeological seeds were analysed for this study.

### Modern seed material

Modern seed material was collected from 13 of the most representative and best preserved populations of *V. sylvestris* present in Sardinia (Fig. 4). The wild grapes were sampled along riverbanks, colluvial sites and hilly damp slopes.



**Fig. 3** Waterlogged archaeological grape pips from Wells N, U and V



In addition, 37 traditional cultivars grown in central-west and southern Sardinia were collected and selected from areas closest to the archaeological site of Sa Osa to evaluate the potential relationship between the cultivars and archaeological seeds (Fig. 4). Two Italian cultivars of *V. vinifera* (Sangiovese and Trebbiano Toscano), and two French ones (Alicante and Pinot Bianco), were added to the study.

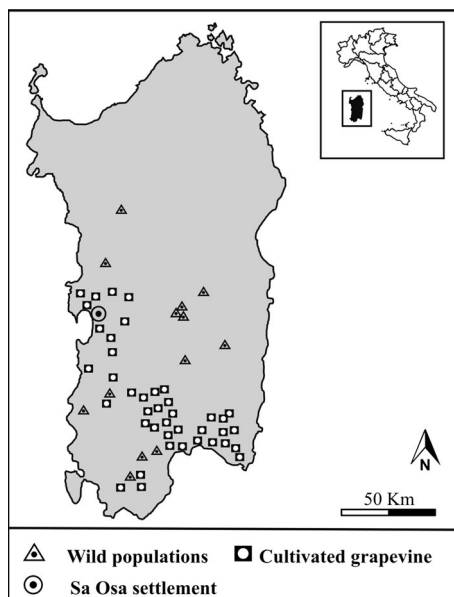
Finally, seeds from 25 wild plants that were grown by the Agricultural Research Agency of Sardinia (AGRIS) for five years through propagation by cuttings were also sampled.

Grapes were collected at the time of maximum sugar concentration in the pulp and therefore ripeness, which corresponds to complete morphological development, and a visual evaluation of the seed colour was used as an index of maturity (Ristic and Iland 2005; Failla 2007). To obtain samples that sufficiently represented the morphometric variability of batches of seeds, ten bunches of grapes from each cultivar were collected from ten different individual

vines (Guarino et al. 1995; Bacchetta et al. 2008a). Then between 10 and 30 grapes were selected from the central part of each bunch depending on the bunch and grape morphology (Orrù et al. 2013). According to the descriptive list of *Vitis* species and varieties, undeveloped (stenospermocarpic) seeds were not considered (OIV 2009).

#### Seed image analysis

Digital images of the modern and archaeological seeds were acquired using an Epson GT 15000 flatbed scanner with a digital resolution of 400 dpi for a scanning area less than  $1,024 \times 1,024$  pixels. The seed samples consisting of 100 seeds were randomly chosen from the original seed batches, but when the original accession was less than 100 units, the analysis was done on the whole seed batch. Image acquisition was performed before drying the seeds at 15 °C at 15 % R.H. to avoid variations in dimension, shape and colour. To characterise the variability of the modern seeds, the seed samples were scanned three times and randomly arranged on the surface of the flatbed scanner each time. The digital images of the seeds were processed and analysed using the KS-400 V3.0 software package (Carl Zeiss Vision, Oberkochen, Germany). A macro that was specially developed to characterise wild seeds (Matana et al. 2008) and later adjusted to measure 20 additional morphometric seed features (Bacchetta et al. 2008b) was adapted to perform the entire analysis procedure automatically to reduce the time taken and decrease the number of contextual mistakes during the analysis process (Grillo et al. 2010). This macro was further enhanced by adding algorithms that computed elliptic Fourier descriptors (EFDs) for each analysed seed, which increased the number of discriminant parameters (Orrù et al. 2012). This method allows description of the outline of the seed as an array of complex numbers which correspond to the pixel positions on the seed outline. So, from the seed apex, which was defined as the starting point in a Cartesian system, chain codes are generated. A chain code is a lossless compression algorithm for binary images. The basic principle of chain codes is to separately encode each connected component (pixel) in the image. The encoder then moves



**Fig. 4** Distributions of selected wild grape populations and cultivars of *Vitis vinifera*

along the outline of the image and, at each step, transmits a symbol representing the direction of this movement. This continues until the encoder returns to the starting position. This method is based on separate Fourier decompositions of the incremental changes of the X and Y coordinates as a function of the cumulative length along the outline (Kuhl and Giardina 1982). Each harmonic ( $n$ ) corresponds to four coefficients ( $an$ ,  $bn$ ,  $cn$  and  $dn$ ) defining the ellipse in the XY plane. The coefficients of the first harmonic, describing the best fitting ellipse of the outlines, are used to standardize size (surface area) and to orientate seeds (Terral et al. 2010). According to Terral et al. (2010), when using a number of harmonics for an optimal description of seed outlines, in order to minimize the measurement errors and to optimize the efficiency of shape reconstruction, 20 harmonics were used to define the seed outlines, obtaining a further 80 parameters which are useful to discriminate between the studied seeds (Orrù et al. 2012).

A total of 98 morphometric characters were measured on 98,338 grape pips (Table 2).

### Statistical analysis

The data obtained from the accessions of modern wild and cultivated and archaeological grape pips were used to establish a database of morphometric grape pip features (Bacchetta et al. 2009; Orrù et al. 2012, 2013). The data were statistically analysed by applying the stepwise LDA

**Table 2** List of 18 morphometric features measured on seeds, excluding the 80 Elliptic Fourier Descriptors (EFDs) calculated according to Hâruta (2011)

| Feature               | Abbreviation        | Description   |
|-----------------------|---------------------|---|
| Area                  | $A$                 | Seed area (mm <sup>2</sup> )  |
| Perimeter             | $P$                 | Seed perimeter (mm)   |
| Convex perimeter      | $P_{conv}$          | Convex perimeter of the seed (mm)                                     |
| Crofton perimeter     | $P_{Crof}$          | Crofton perimeter of the seed (mm)                                    |
| Perimeter ratio       | $P_{conv}/P_{Crof}$ | Ratio between $P_{conv}$ and $P_{Crof}$                               |
| Maximum diameter      | $D_{max}$           | Maximum diameter of the seed (mm)                                     |
| Minimum diameter      | $D_{min}$           | Minimum diameter of the seed (mm)                                     |
| Feret ratio           | $D_{min}/D_{max}$   | Ratio between $D_{min}$ and $D_{max}$                                 |
| Maximum ellipse axis  | $EA_{max}$          | Maximum axis of an ellipse with equivalent area (mm)                  |
| Minimum ellipse axis  | $EA_{min}$          | Minimum axis of an ellipse with equivalent area (mm)                  |
| Shape Factor          | $Sf$                | Seed shape descriptor = $(4\pi A)/P^2$ (normalized value)             |
| Roundness Factor      | $Rf$                | Seed roundness descriptor = $(4A)/(\pi D_{max}^2)$ (normalized value) |
| Eq. circular diameter | $Ecd$               | Diameter of a circle with equivalent area (mm)                        |
| Fibrelength           | $F$                 | Seed length along the fibre axis                                      |
| Curl degree           | $C$                 | Ratio between $D_{max}$ and $F$                                       |
| Convexity degree      | $Conv$              | Ratio between $P_{Crof}$ and $P$                                      |
| Solidity degree       | $Sol$               | Ratio between $A$ and convex area                                     |
| Compactness degree    | $Com$               | Seed compactness descriptor = $[\sqrt{(4/\pi)A}]/D_{max}$             |

method and by using the SPSS software package release 15.0 (SPSS Inc. 2006) to compare the modern cultivars with the archaeological seeds, which were considered as unidentified specimens.

This approach is commonly used to classify and identify unknown groups that are characterised by quantitative and qualitative variables (Bacchetta et al. 2008b, 2009, 2010, 2011b; Venora et al. 2009a, b; Grillo et al. 2010; Orrù et al. 2012, 2013; Smykalova et al. 2011). This is accomplished by determining the combination of predictor variables while simultaneously minimising the within-class distance and maximising the between-class distance to achieve maximum discrimination (Hastie et al. 2001; Holden et al. 2011).

The stepwise method identifies and selects the best features and uses them to identify seed samples using three statistical variables: *Tolerance*, *F-to-enter* and *F-to-remove*. The *Tolerance* value indicates the proportion of the variance of a variable that is not accounted for by other independent variables in the equation. A variable with extremely low *Tolerance* values provides little information to the model. The *F-to-enter* and *F-to-remove* values define the power of each variable in the model and describe what happens if a variable is either inserted or removed from the current model (Bacchetta et al. 2010). This method starts with a model that does not include any variables. At each step, the variable with the largest *F-to-enter* value that exceeds the selected entry criteria ( $F \geq 3.84$ ) is added to the model. The variables omitted from the analysis at the last step have *F-to-enter* values smaller than 3.84 and are not added. The process is automatically stopped when no remaining variables are able to increase the discrimination of the method (Venora et al. 2009b). Finally, a cross-validation procedure is applied to verify the performance of the identification system by testing individual unknown cases and classifying them on the basis of all others. This procedure, also called rotation estimation (Picard and Cook 1984; Kohavi 1995), was applied, both to evaluate the performance and to validate any classifier. The validation procedure used here is the leave-one-out cross-validation (LOOCV). It involves using a single case from the original sample set as the validation dataset, and the remaining cases as the training set. Each case is classified into a group according to the classification functions computed from all the data, except the case being classified. The proportion of misclassified cases after removing the effect of each case one at a time is the leave-one-out estimate of misclassification (SPSS 2006).

All the raw data were standardized before starting any statistical work. Moreover, in order to evaluate the quality of the discriminant functions achieved for each statistical comparison, *Wilks' Lambda*, *Eigenvalues*, the percentage of explained variance, *Chi square* and the standardized

canonical discriminant function coefficients (SCDFCs) were computed.

## Results

Each batch of archaeological pips was compared with all of the cultivated and wild grape pips. From the comparison between the batches of archaeological seeds, which were considered individually and added to the classifier as unknown groups, and the modern *V. vinifera* and *V. sylvestris*, an overall percentage of 96 % were correctly identified. The archaeological pips from Well N were identified as *V. vinifera* in 95.3 % of the cases, those from Well U were equally distributed between *V. vinifera* and *V. sylvestris*, and the ones from Well V were identified as *V. sylvestris* in 66.3 % of the cases (Table 3). A comparison of the archaeological pips from Wells U and V was done, and an overall percentage of 67.8 % were correctly identified, with individual performances of correct identification of 59 and 73.7 % for Wells U and V respectively (data not shown). This shows that these two batches of seeds are distinguishable. Including the archaeological seeds from Well N, a slight improvement in the classification between Wells U and V was observed. In this case, an overall correct classification of 91.9 % was achieved; however, the high performance of the Well N seeds was a contributing factor. This group was perfectly identified, while the seeds from Wells U and V were correctly classified in 74.5 and 63 % of the cases, respectively, with incorrect attribution only between them (data not shown). The graphical representation of the LDA of the seeds from the three wells is shown in Fig. 5. The two discriminant functions achieved by the stepwise process only included 17 of the 98 measured morphometric features. The 17 chosen features are shown with the respective values of *F-to-remove*, *Wilks' Lambda* and the SCDFCs that can be used to compute the canonical variable score of the two discriminant functions, in Table 4. The very low values of *Wilks' Lambda* indicate strong differences between the groups. Moreover, for the

two discriminant functions, the relative values of *Wilks' Lambda*, *Chi square*, *Eigenvalues* and the percentage of explained variance are given in Table 5. Although both discriminant functions are necessary to reach the best differentiation between the groups, the high values of *Eigenvalues* for the first discriminant function, together with the very high percentage of explained variance, *Wilks' Lambda* and the high *Chi square*, explain the great importance of this discriminant function for the second one.

Considering that only Wells U and V showed similarities to the wild populations (Table 3), the archaeological seeds, which were considered as unknown specimens, were compared with the pips of wild grape populations. The batches of seeds from Well U displayed similarities of 50 and 49.5 % to the Mandas and Giave populations respectively (Table 6); likewise, those from Well V, which were also considered as unknown, showed similarities of 33.3 and 63.3 % to the Mandas and Giave populations, respectively (Table 6).

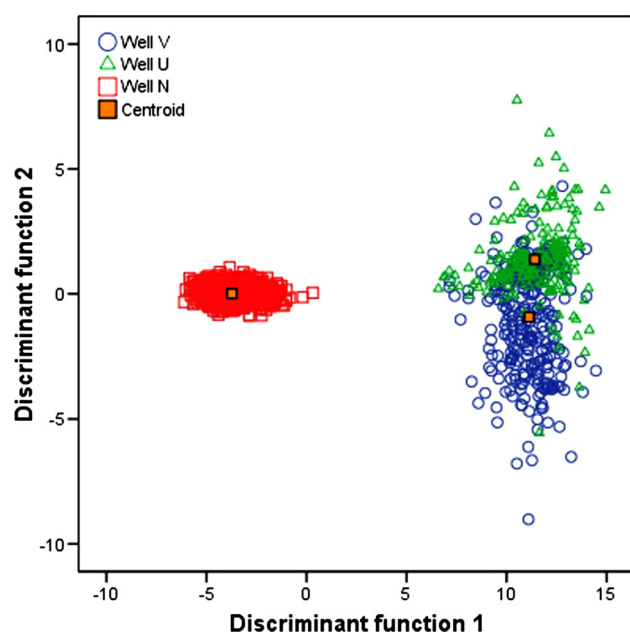
Similarly, considering that only the pips from Wells N and U showed similarities to cultivars (Table 3), the seeds from these two wells were compared with those of vines of particular grape colours. The highest percentage of the pips from Well N, considered as unknown seeds, was identified as being from white grapes (61.2 %), but 38.8 % of the cases were classified as being from black grapes (data not shown); whereas the whole batch from Well U was identified as white grapes (data not shown).

Finally, widening the database to also include seeds from cultivated wild populations, the pips from Wells U and V showed a strong relationship with cultivated wild populations (Table 7). The comparison of the pips from Well U indicate relationships with Population 218 (Siliqua) in 50 % of the cases and with Population G42 (Santadi) in 46.5 % of the cases (Table 7). Similar results were obtained after comparing the pips from Well V with the cultivated wild populations. In this case, similarities with Population 218 and with Population G42 were detected in 66.3 and 33.3 % of the cases, respectively (Table 7).

**Table 3** Identification percentage among the three batches of archaeological pips of *V. vinifera* and *V. sylvestris* from Wells N, U and V (considered unknown seeds)

The numbers of pips that were analysed are in brackets

|   | <i>V. vinifera</i> ssp. <i>vinifera</i> | <i>V. vinifera</i> ssp. <i>sylvestris</i> | Total        |
|---|---|---|--------------|
| <i>V. vinifera</i> ssp. <i>vinifera</i>   | 90.1 (77,428)                           | 9.9 (8,498)                               | 100 (85,926) |
| <i>V. vinifera</i> ssp. <i>sylvestris</i> | 20.3 (740)                              | 79.7 (2,912)                              | 100 (3,652)  |
| Well N archaeol. seeds                    | 95.3 (1,438)                            | 4.7 (71)                                  | 100 (1,509)  |
| Well U archaeol. seeds                    | 50.0 (100)                              | 50.0 (100)                                | 100 (200)    |
| Well V archaeol. seeds                    | 33.7 (101)                              | 66.3 (199)                                | 100 (300)    |
| Overall                                   |   |   | 96 (91,587)  |



**Fig. 5** Graphic representation of the discriminating function scores for wells N, U and V

**Table 4** Key parameters selected by the stepwise LDA model to discriminate between the pips from the three archaeological wells and the relative statistic values of *F-to-remove*, *Wilks' Lambda* and *Standardized Canonical Discriminant Function Coefficients (SCDFC)*

| Feature                                    | <i>F-to-remove</i> | <i>Wilks' Lambda</i> | SCDFC    |          |
|--|--------------------|----------------------|----------|----------|
|  |                    |                      | Funct. 1 | Funct. 2 |
| <i>A</i>                                   | 59,331             | 0.019                | -0.948   | 0.545    |
| <i>Ecd</i>                                 | 130,851            | 0.020                | 2.857    | -1.895   |
| <i>D<sub>max</sub></i>                     | 37,434             | 0.018                | 0.898    | 0.486    |
| <i>D<sub>min</sub></i>                     | 28,367             | 0.018                | -0.774   | 0.937    |
| <i>EA<sub>max</sub></i>                    | 33,576             | 0.018                | -1.260   | 0.479    |
| <i>P<sub>conv</sub> / P<sub>Crof</sub></i> | 36,854             | 0.018                | 0.165    | 0.255    |
| <i>a2</i>                                  | 8,109              | 0.018                | 0.108    | -0.631   |
| <i>a4</i>                                  | 50,624             | 0.019                | -0.515   | 2.839    |
| <i>a5</i>                                  | 31,211             | 0.018                | -0.109   | 0.417    |
| <i>a8</i>                                  | 15,838             | 0.018                | 0.092    | -0.348   |
| <i>b3</i>                                  | 4,613              | 0.018                | -0.017   | 0.136    |
| <i>c2</i>                                  | 5,742              | 0.018                | -0.001   | 0.157    |
| <i>d1</i>                                  | 131,832            | 0.020                | 0.729    | -1.133   |
| <i>d2</i>                                  | 39,770             | 0.018                | -0.256   | 1.628    |
| <i>d5</i>                                  | 74,021             | 0.019                | -0.043   | 0.798    |
| <i>d9</i>                                  | 8,815              | 0.018                | 0.011    | -0.275   |
| <i>d14</i>                                 | 5,510              | 0.018                | 0.030    | 0.171    |

## Discussion

The availability of substantial numbers of waterlogged grape pips together with innovative technological resources

have allowed us to compare, from the morphometric point of view, the MBA and LBA Age archaeological pips from Sa Osa with modern cultivars and wild populations collected in central-west and southern Sardinia.

The LBA grape pips from Well N showed a greater similarity to current grapevine cultivars, those from the MBA from Well U were intermediate domesticated forms, and those from Well V showed higher percentages of similarity with the tested wild grape populations. The close similarity of the archaeological pips to *V. vinifera* cultivars and the wild *V. sylvestris* populations of Sardinia might suggest local viticulture, however, the possibility that the grapevine cultivation techniques were learned from a neighbouring settlement should not be forgotten. Morphological analysis revealed a certain level of similarity between the pips from Wells U and V, so it is probable that they belonged to the same grapevine accession/population, as confirmed by <sup>14</sup>C dating of both wells.

The comparisons also demonstrated that the seeds from Wells N and U might be related to white grape varieties. Although a considerable percentage of mis-attribution exists between white and black grapes, these results agree well with those of Orrù et al. (2012). In order to identify a narrower group of *V. vinifera* to which the archaeological pips could be a closer match, they compared the pips from the two archaeological sites with the *V. vinifera* cultivars which were clustered for grape colour. They explained that the greater similarity of the archaeological pips to *V. vinifera* cultivars rather than to *V. sylvestris* populations, and especially to white grapes rather than black grape cultivars, could validate the idea that white grapes were probably already used at 1600–1200 BC in southern Sardinia. Therefore, it might be not a chance that white grapes are still traditionally grown today in that area to produce famous wines, such as Vernaccia and Malvasia. Proving that a relationship between pip morphology and grape colour exists, the achievements of this study may confirm that the archaeological pips belong to cultivated varieties. Many studies indicate that numerous genetic changes could have occurred during the domestication process, which may also have affected grape colour (Hancock 2004; This et al. 2007; Brown et al. 2009; Fournier-Level et al. 2010). Genetic analysis has revealed that the appearance of white grapes is related to the domestication process, which would have caused a mutation in the gene *VvMybA* and varied the content of anthocyanin in grapes from cultivated vines (Kobayashi et al. 2005; Cadle-Davidson and Owens 2008; Fournier-Level et al. 2010). In wild populations, even at an intraspecific genetic level, there are no changes in grape colour. Therefore, all modern white grapes would have been derived from wild progenitors which became domesticated (Cadle-Davidson and Owens 2008). When the pips of cultivated



**Table 5** Statistical values of *Wilks' Lambda*, *Chi square*, *Eigenvalues* and the percentages of explained variance of the two discriminant functions computed by stepwise LDA to discriminate between the archaeological pips from the three wells

|                         | <i>Wilks' Lambda</i> | <i>Chi-square</i> | <i>Eigenvalue</i> | % of variance | % cumulative |
|-------------------------|----------------------|-------------------|-------------------|---------------|--------------|
| Discriminant function 1 | 0.018                | 8,065.030         | 41.927            | 99.2          | 99.2         |
| Discriminant function 2 | 0.758                | 553.532           | 0.319             | 0.8           | 100          |

modern and wild grapevines were also considered in the morphological comparisons, only the archaeological pips from the MBA were similar to these accessions.

Most probably the wild grape vines cultivated by AGRIS through propagation by cuttings produced seeds with a similar morphology to the archaeological pips. It is possible to assume that the first vine growers in the MBA used the same cultivation technique (Zohary 1995; Zohary et al. 2012). Based on the acquired data, it could be hypothesised that during the evolution of pips in the MBA, intermediate forms between wild and cultivated plants may have existed that represented early stages of the domestication of grapevines. The results of the present study suggest that the selection and domestication processes of the wild grape may have started during the MBA. This theory is also supported by the results of recent pollen analyses performed on lake material from the Lago di Mistras, which is located 7 km from the site of Sa Osa (Di Rita and Melis 2013).

The *Vitis* pollen recorded from core MTR1-3 dates from some time between the MBA and the Punic period (3950–2050 cal BP) and suggests local viticulture (Di Rita and Melis 2013). Considering that *Vitis* pollen has a low dispersion rate (Turner and Brown 2004) and that soils in this area are highly saline and not suitable for the growth of wild grapes, it is possible that the pollen found in the Lago di Mistras originates from cultivated grapevines that would have been planted in areas facing the gulf of Oristano. Moreover, the surveys performed in this part of Sardinia indicate that the nearest wild grape populations are currently located 25 km from the sites of Mistras and Sa Osa. Therefore, it is unlikely that the pollen of these plants could have reached the coast. Furthermore, a recent pollen study of Well N revealed a high percentage (3.5 %) of grape pollen (Sabato 2014).

Our exploratory surveys performed over the entire area of the gulf of Oristano and along the river Tirso confirm the absence of wild grapevines and the existence of several vines that had escaped from vineyards. This allowed us to hypothesise that wild grapes were also absent from this area in the distant past. The absence of wild grape from this area of Sardinia is most probably related to the soil characteristics which are predominately derived from limestone. Moreover this area is characterised by continuous morphological changes from wind, marine and dynamic

fluvial activities, all of which make it difficult for wild grape to grow. For this reason, we hypothesise that the grape pollen found in Well N did not come from local populations of wild grape, but that cultivated grapevines were present near the Sa Osa settlement during the LBA. More evidence of viticulture in Sardinia during the MBA was provided by a recent anthracological (charcoal) study performed at the Domus de Janas IV, which is situated in the territory of Usini (Sassari) in northern Sardinia. It revealed the presence of a large amount of charred *V. vinifera* wood associated with grape pip remains from a hearth dating back to the beginning of the MBA (ca. 1780–1630 BC) (Celant 2010).

The presence of *V. vinifera* charcoal in archaeological contexts is a rather rare event. Charcoal of grapevine has never been found in hearths at Neolithic sites in the Middle East, despite the abundance of wild grapes in the surrounding areas, according to archaeobotanical studies (Willcox 1991). The first presence of charcoal of *Vitis* is recorded only from the second half of the 3rd millennium, a period in which grapevine domestication has been recorded. For this reason, it is plausible that the presence of *V. vinifera* charcoal at the archaeological sites may represent pruning remains of grapevine (Thiébaud 1989; Bouby and Marinval 2001; Miller 2008).

A recent study has provided numerous insights into the subsistence economy and agriculture practised in Sardinia during the BA (Ucchesu et al. 2014). Archaeobotanical data show the presence of cereals and legumes which confirm the diversified agricultural practices of the Nuragic communities (Ucchesu et al. 2014). Therefore, it is hardly surprising that these communities may also have experimented with the cultivation of the grapevine, which may have been stimulated by new agricultural techniques brought by foreigners. On the basis of these achievements, we might hypothesize that the Nuragic society started a sort of selection of wild grapevines during the MBA, developing into an early stage of grape cultivation and domestication in the LBA.

## Conclusions

The discovery of a large quantity of grape pips preserved in waterlogged contexts at the site of Sa Osa have allowed us

**Table 6** Correct classification percentages among populations of *V. sylvestris* and archaeological seeds from Wells U and V (Sa Osa)

|                 | Mandas 1   | Laconi 1   | Laconi 3   | Siliqua 1  | Santadi 2  | Uta 3      | Perdas     | Flumini-<br>maggiore | Sibiri     | Sos Molinos | Giave      | Laconi 2   | Arizzo     | Total         |
|-----------------|------------|------------|------------|------------|------------|------------|------------|----------------------|------------|-------------|------------|------------|------------|---------------|
| Mandas 1        | 64.4 (57)  | 1.1 (1)    | 2.2 (2)    | 3.3 (3)    | 4.4 (4)    | 2.2 (2)    | 6.7 (6)    | -                    | 1.1 (1)    | 10 (9)      | 2.2 (3)    | 1.1 (1)    | 1.1 (1)    | 100 (90)      |
| Laconi 1        | -          | 64.5 (185) | 6.7 (23)   | 5.4 (19)   | 0.3 (1)    | 2.7 (8)    | 4.0 (13)   | 3.3 (10)             | -          | 0.7 (2)     | 0.7 (3)    | 7.4 (22)   | 4.3 (13)   | 100 (299)     |
| Laconi 3        | -          | 14.2 (47)  | 51.5 (142) | -          | 5.8 (18)   | 9.2 (28)   | 1.0 (4)    | 9.5 (30)             | 2.0 (6)    | -           | -          | 5.1 (15)   | 1.7 (5)    | 100 (295)     |
| Siliqua 1       | -          | 3.0 (9)    | 0.3 (1)    | 83.3 (248) | -          | 2.0 (6)    | 0.3 (1)    | -                    | -          | 3.7 (12)    | 1.3 (4)    | 1.7 (5)    | 4.3 (14)   | 100 (300)     |
| Santadi 2       | 0.3 (2)    | 1.3 (4)    | 5.0 (16)   | 0.3 (1)    | 51.7 (145) | 13.8 (41)  | 1.0 (3)    | 9.1 (30)             | 13.8 (45)  | 2.7 (8)     | 1.0 (3)    | -          | -          | 100 (298)     |
| Uta 3           | -          | 2.0 (6)    | 8.1 (30)   | 1.0 (3)    | 16.5 (51)  | 54.2 (151) | 2.0 (6)    | 5.4 (17)             | 4.7 (15)   | 1.0 (3)     | 0.3 (1)    | 4.0 (12)   | 0.7 (2)    | 100 (297)     |
| Perdas          | 2.8 (8)    | 1.0 (4)    | 2.8 (8)    | 3.1 (9)    | 2.1 (6)    | 1.4 (4)    | 53.8 (149) | 6.9 (22)             | 1.4 (4)    | 2.8 (10)    | 5.5 (17)   | 6.9 (21)   | 9.7 (28)   | 100 (290)     |
| Fluminimaggiore | 2.0 (6)    | 4.7 (16)   | 4.7 (15)   | 1.0 (3)    | 9.4 (28)   | 10.4 (32)  | 9.4 (27)   | 40.1 (114)           | 3.0 (9)    | -           | 3.0 (9)    | 8.4 (25)   | 3.7 (13)   | 100 (297)     |
| Sibiri          | 0.7 (2)    | 0.3 (1)    | 5.0 (16)   | -          | 12.4 (38)  | 7.4 (22)   | 1.7 (5)    | 3.0 (8)              | 60.2 (179) | 5.7 (17)    | 1.7 (5)    | 0.7 (2)    | 1.3 (4)    | 100 (299)     |
| Sos Molinos     | 7.8 (23)   | 4.1 (12)   | 1.0 (3)    | 3.1 (10)   | 0.3 (2)    | 1.0 (3)    | 1.0 (3)    | -                    | 6.5 (20)   | 68.6 (196)  | 3.4 (12)   | 1.4 (5)    | 1.7 (4)    | 100 (293)     |
| Giave           | 0.3 (1)    | 0.7 (2)    | 0.7 (2)    | 1.0 (3)    | 0.3 (1)    | 1.3 (4)    | 3.0 (11)   | 0.3 (2)              | 0.7 (2)    | 3.0 (10)    | 79.3 (233) | 0.3 (1)    | 9.0 (27)   | 100 (299)     |
| Laconi 2        | 1.0 (3)    | 10.4 (32)  | 6.4 (19)   | 2.7 (9)    | 0.7 (2)    | 2.4 (8)    | 10.4 (36)  | 4.4 (14)             | -          | 0.7 (2)     | 1.7 (5)    | 51.9 (145) | 7.4 (22)   | 100 (297)     |
| Arizzo          | -          | 6.4 (19)   | 2.0 (6)    | 4.0 (12)   | 1.0 (3)    | 1.0 (5)    | 10.1 (32)  | 3.0 (10)             | -          | 1.3 (4)     | 10.1 (32)  | 8.1 (25)   | 53.0 (150) | 100 (298)     |
| Well V          | 33.3 (100) | -          | -          | -          | -          | -          | -          | -                    | -          | -           | 63.3 (190) | -          | 3.3 (10)   | 100 (300)     |
| Well U          | 50.0 (100) | -          | -          | -          | -          | -          | -          | -                    | -          | -           | 49.5 (99)  | -          | 0.5 (1)    | 100 (200)     |
| Overall         |            |            |            |            |            |            |            |                      |            |             |            |            |            | 57.3% (4,152) |

The numbers of pips that were analysed are in brackets

**Table 7** Correct classification percentage among wild cultivated, wild population of *V. sylvestris* and archaeological pips from Wells U and V of the Sa Osa settlement

| Populations      | Classification (%) | Archaeo-seeds Sa Osa |            | Total          |
|------------------|--------------------|----------------------|------------|----------------|
|                  |                    | Well U               | Well V     |                |
| Wild cropped     |                    |                      |            | 100 (657)      |
| G16              | 56.0 (368)         | -                    | -          | 100 (300)      |
| G21              | 48.3 (145)         | -                    | -          | 100 (102)      |
| G23              | 57.8 (59)          | -                    | -          | 100 (709)      |
| G29              | 65.6 (465)         | -                    | -          | 100 (48)       |
| G31              | 18.8 (9)           | -                    | -          | 100 (300)      |
| G36              | 81.7 (245)         | -                    | -          | 100 (300)      |
| G37              | 51.7 (155)         | -                    | -          | 100 (108)      |
| G39              | 12.0 (13)          | -                    | -          | 100 (300)      |
| G42 (Santadi)    | 80.0 (240)         | 46.5 (93)            | 33.3 (100) | 100 (300)      |
| G47              | 73.3 (220)         | -                    | -          | 100 (126)      |
| G49              | 38.89 (49)         | -                    | -          | 100 (300)      |
| 213              | 29.7 (89)          | -                    | -          | 100 (300)      |
| 214              | 35.3 (106)         | -                    | -          | 100 (300)      |
| 215              | 38.7 (116)         | -                    | -          | 100 (300)      |
| 216              | 48.3 (145)         | -                    | -          | 100 (144)      |
| 217              | 13.9 (20)          | -                    | -          | 100 (54)       |
| 218 (Siliqua)    | 87.0 (47)          | 50.0 (100)           | 66.3 (199) | 100 (300)      |
| 219              | 58.3 (175)         | -                    | -          | 100 (300)      |
| 220              | 55.0 (155)         | -                    | -          | 100 (270)      |
| 221              | 61.5 (166)         | -                    | -          | 100 (300)      |
| 222              | 53.0 (159)         | -                    | -          | 100 (300)      |
| 223              | 58.0 (174)         | -                    | -          | 100 (300)      |
| 224              | 74.3 (223)         | -                    | -          | 100 (300)      |
| 225              | 53.0 (159)         | -                    | 0.3 (1)    | 100 (33)       |
| 226              | 90.9 (30)          | 3.5 (7)              | -          | 100 (200)      |
| Wild populations |                    |                      |            |                |
| Mandas 1         | 37.8 (34)          | -                    | -          | 100 (90)       |
| Laconi 1         | 52.2 (156)         | -                    | -          | 100 (299)      |
| Laconi 3         | 35.6 (105)         | -                    | -          | 100 (295)      |
| Siliqua 1        | 69 (207)           | -                    | -          | 100 (300)      |
| Santadi 2        | 31.5 (94)          | -                    | -          | 100 (298)      |
| Uta 3            | 34.3 (102)         | -                    | -          | 100 (297)      |
| Perdas           | 28.3 (82)          | -                    | -          | 100 (290)      |
| Fluminimaggiore  | 27.3 (81)          | -                    | -          | 100 (297)      |
| Sibiri           | 40.8 (122)         | -                    | -          | 100 (299)      |
| Sos Molinos      | 53.6 (157)         | -                    | -          | 100 (293)      |
| Giave            | 72.9 (218)         | -                    | -          | 100 (299)      |
| Laconi 2         | 27.9 (83)          | -                    | -          | 100 (297)      |
| Aritzo           | 39.3 (117)         | -                    | -          | 100 (298)      |
| Overall          |                    |                      |            | 48.9% (10,603) |

The numbers of seeds that were analysed are in brackets

to investigate the domestication status of grapes during the BA in Sardinia. The LDA analysis showed that the archaeological material had affinities close to wild grape and *V. vinifera* cultivars in Sardinia; in particular, the archaeological pips from the LBA showed a greater affinity to grapevine cultivars, whereas those from the MBA were intermediate domestication forms. Moreover, the analysis demonstrated that the archaeological pips from the MBA

and the LBA most probably both belonged to white grape varieties. This could be considered as evidence of a state of domestication because the grapes would have been intentionally selected. We do not know if the cultivation techniques were introduced from external sources or not, but we can speculate that this could have occurred through contact with Mycenaean or Minoan traders. Regardless, the results of this work provide evidence that cultivated grapevines were present in Sardinia during the LBA.

Much archaeobotanical and genetic evidence suggests that there may have been independent secondary sites of grape domestication in the western Mediterranean area, however the evidence of cultivated grape remains is still not completely established (Rivera-Núñez and Walker 1989; Grassi et al. 2003, 2008; Sefc et al. 2003; Arroyo-Garcia et al. 2006). Sardinia could be a valid site of secondary domestication of grapes. This is suggested by the presence of numerous wild grape populations, which still remain unaltered due to the geological and climatic conditions of Sardinia, and the presence of pristine habitats in the island. Therefore, it is reasonable to hypothesize that BA agriculturists may have intentionally selected wild grapes and experimented with their cultivation. Consequently, they would have produced grapevines that could be still related to modern Sardinian autochthonous cultivars. Even if we are still far from having a full understanding of the process of the domestication of grapevines, we hope that this research will stimulate new investigations in this field.

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