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Dipartimento di Scienze della Vita e dell'Ambiente

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Ecology, Ecophysiology and Morpho-colorimetric analysis of wild grapevines populations of Sardinia and relationships with some autochthonous cultivars

Presentata: Martino Orrù
Coordinatore Dottorato Prof. Gianluigi Bacchetta
Relatori: Prof. Gianluigi Bacchetta
Dott. Gianni Lovicu
Correlatore: Dott. Gianfranco Venora

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Summary

…a chi ha saputo trasformare ogni goccia del proprio lavoro in buon vino.

…ai custodi della cultura e della tradizione.

1. Introduction

1.1 The *Vitis* L. genus

The first fossils (pollen and leaves) belonging to the *Vitis* L. genus, dated around the Palaeogene period of the Cenozoic Era (65.95-28.4 million of years ago), have been classified as *Vitis sezannensis* Saporta, *V. ampelophyllum* Lesquereux (Italy) and *V. balbiani* which have been found respectively in France, Italy and in several other places in Europe. All the *Vitis* show morphological features which are deeply different from the present types (Fregoni 1991) (Fig. 1).

The most conspicuous ancestral grapevines' finds have been dated around the Miocene (25 millions of years ago), when the mild climate fostered the spread of grapevines also around areas which do not exist anymore; an evidence of the latter is given by the *V. islandica* Heer (Iceland), the *V. olriki* Heer (Greenland), the *V. crenata* Heer (Alaska), the *V. britannica* Heer (United Kingdom and other countries in Northern Europe), the *V. teutonica* Braun (Germany, Switzerland, France), the *V. hookeri* Heer (Germany, Switzerland, France), and the *V. vivarensis* Boul (Germany, Switzerland, France); they are all characterized by a morphological similarity with modern grapevines of American origins, while the *V. ludwigii* and the *V. braunii* Lud. (Germany) show leaves with typical transitional morphology, as the Eurasian grapevine (Fregoni 1991; Ocete et al. 2004).

Figure 1. Morphological differents beetwen ancestral and attually *Vitis* L. leaves (from Fregoni 1991).

The other fossil finds, ascribable to the Miocene period, include *V. alaskana* Heer (Alaska), *V. arctica* Heer (Greenland) *V. braunii* Ludwig (Germany) e *V. dutaillyii* Munier

(Ireland) (Dalmasso 1957; Dalmasso G and Eynard I. 1979). From a phylogenetic point of view, the *V. praevinifera* Saporta (France) is deemed the ancestral grapevine with the closest relation with modern European grapevine, while the *V. subintegra* Saporta, *V. salvorum* Saporta and *V. aussoniae* Gandin found in France and attributable to the Pliocene period (7 millions of years ago) show a morphology of the leaf very much similar to the fossil rests of the *V. vinifera* L. found in the whole Northern hemisphere (Dunque and Barrau 2005; Fregoni 1991; Dalmasso 1957).

During the glaciations of Quaternary in the Pliocene period (1,806 – 0,126 years), the change in climatic conditions has allowed the development of huge icecaps having their starting point in the Northern hemisphere (Jansen et al 2000). During the short interglacial periods ice caps subjected to substantial reduction due to the temperature rise (similar or warmer than the present ones) (Frogley et al. 1999). These intermediate periods have determined the isolation of those populations of grapevine, producing the consequential genetic diversification through allotropic speciation (Willis and Niklas, 2011) how *Vitis vinifera* L. subspecies *sylvestris* (Gmelin) Hegi (for convenience purpose from here reported as *V. v. sylvestris*)was found to be sister to all Asian species and to be one of the many oldest living Eurasian species (Zecca et al. 2012).

In the interglacial periods important re-distributional phenomenon happened repeatedly, as well as the isolation of plants in areas propitious to adverse conditions (shelter) which has been fundamental to the evolution of the species (Bennett 1990, 1997). The Caucasus, the Iberian Peninsula, Italy and the Sardinian Island could have had a key-role in protecting the genetic diversity of the *V. v. sylvestris* and thus allowed the quick colonization of Central and Northern Europe, in the post-glacial period of those places which were chosen by the species (Grassi et al. 2008).

1.2 Habitat and distribution of the wild grapevines in the Old World

In the Northern hemisphere, several vegetable fossil finds have been attributed to the *Vitis* genus (around 40 species) probably from the Mesozoic period (65-7 millions of years) (Fregoni 1991). The great majority of these species has now disappeared, while some of them, thanks to the climatic shelters on the Mediterranean Sea and in the south-eastern areas of the United States, in Eastern Asia and in the Caucasus, have managed to survive, thus creating two different centres of genetic variability of the *Vitaceae* family. The first include the peninsulas and islands of Mediterranean, Asia Minor and Northern Africa, while the second extends its area from the Black Sea to India, taking up a total area of 6000 square kilometres (Unwin 1991).

Several scholars (historians, botanical agronomists and archaeologists) have been facing the matter of the grapevine's origins over the decades, searching for useful elements in those areas where the culture used to grow spontaneously (Costantini et al 2006). The Caucasian region has been considered for historical and cultural reasons (Hehn 1870: De Candolle 1883) an independent centre of evolution for the grapevine (Costantini et al 2006), because of the presence of a high number of species and varieties of other cultivated plants (wheat, barley, legumes, glax, grape and many other fruits).

Grassi et al. 2006, thanks to the microsatellites in cp-DNA analysis on 418 samples of 78 different populations of wild grapevine coming from the whole Mediterranean basin, stated that the possible centre of origin is in the Caucasian area, while the minor diversity in the haplotype suggest the Iberian peninsula, Central Italy and Sardinia as refugia area.

The climatic refugia (Grassi et al. 2008) and vectors such as men, birds, foxes, bears and turtles (Mabberley 2008) had had a key-role in the re-colonization of a wide range of habitats and soils in the Mediterranean basin and a limited number of places in Central Europe (Grassi et al. 2006). Also to Olmo (1996) the area of distribution of the grapevine has remained

unchanged from the Pliocene period to the Neolithic, including those regions from the Mediterranean to the Caspian Sea. In present times different varieties (eco-types) of wild grapevine grow spontaneously in a vast area between Anatolia and Pakistan (Olmo 1996).

Thus the range of distribution of the wild grapevine thus goes from Northern Africa to Central Europe (Arnold 1998), from 0 to 1000 meters a.s.l., along river shores, on screeds (colluvial sites) of hilly humid slopes and occasionally on coastal sheers and beaches (Ocete et al. 2008). This distribution is deeply fragmented in disjointed micro- or meta-populations formed by few units (Terral et al. 2010), because of the rise of human impact on lowland areas (especially on the western part of the Mediterranean basin); new pests (*Phylloxera vastatrix* Planchon, *Uncinula necator* (Schwein.) Burrill) come from North America about 150 years ago (Grassi et al. 2006; De André et al. 2011) (Fig. 2).

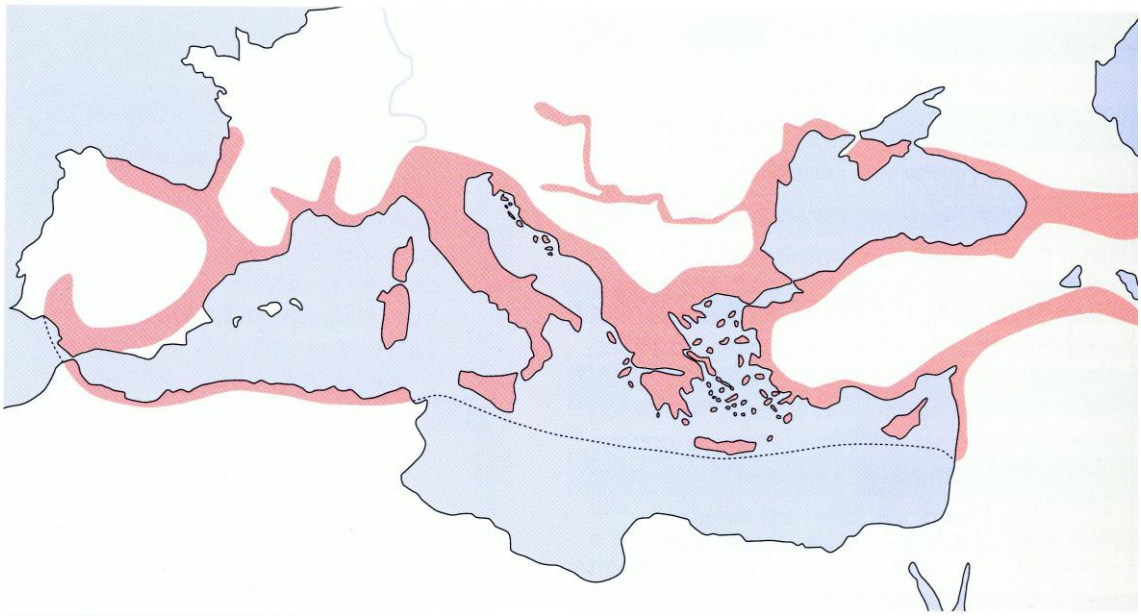

Figure 2. Distribution map of *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel) in Mediterranean basin (from Zohary and Hopf 1993).

The wild grapevine is closely related to humidity and requires high quantities of light (heliophilous) for a good ripeness of the grape itself; for this reason it is always found in nature in well-exposed positions, in woods' borders, near meadows and along edges.

Frequently, wild populations found in natural habitats are considered to be a mixture of wild forms, naturalized cultivated forms and rootstocks escaped from vineyards as well as hybrids derived from spontaneous hybridizations among those species and forms (Laguna 2003; This et al. 2006). Di Vecchi et al. 2009 detected the existence of gene flow between cultivated and wild grapevine, estimating up to 3% of pollen migration between the cultivated vineyards and closely located wild grape populations. These pollen fluxes may have a significant effect on the evolution of those populations (Andrés et al. 2011). Quantification of genetic variation (heterozygosity) in wild grapevine populations analysed in Morocco, Sardinia, Portugal, France or Italy (Grassi et al. 2003; Cunha et al. 2007; Lopes et al. 2009; Zecca et al. 2010; Zinelabine et al. 2010) highlight the low probability of intraspecific hybridization.

The grapevine is an evergreen plant, ligneous and with caduceus leaves, typical of mild climates. To fulfil harmonically its annual cycle it needs an adequate wintry period of cold, to which it gets ready becoming quiescent. The beginning of this process is characterized by a series of physiological adaptations to the harsh temperatures, which show themselves through sprout lignification and leaves' fall. Moreover, the resistance to the wintry cold is relatively limited in comparison to other mild-climates plants; during the vegetative rest, temperatures below –15 °C could in fact provoke irreversible damages to the plants. This fact delimits the area of distribution of both the spontaneous and cultivated wild grapevine strictly to those regions with an average annual temperature which doesn't register less than 10 °C, with a vegetative season (the period when the average daily temperature does not go below the 10 °C) not shorter than 220 days.

As a consequence, the necessity of a cold-enough wintry period limits the area of wild grapevine, and the cultivation of the domestic one, to those regions with an average annual temperature over the 20 °C (Fig. 3).

In the tropical and equatorial areas is nevertheless possible to cultivate the grapevine through specific techniques of pruning and chemical treatments able to remove the quiescent state of the bud (Failla 2007).

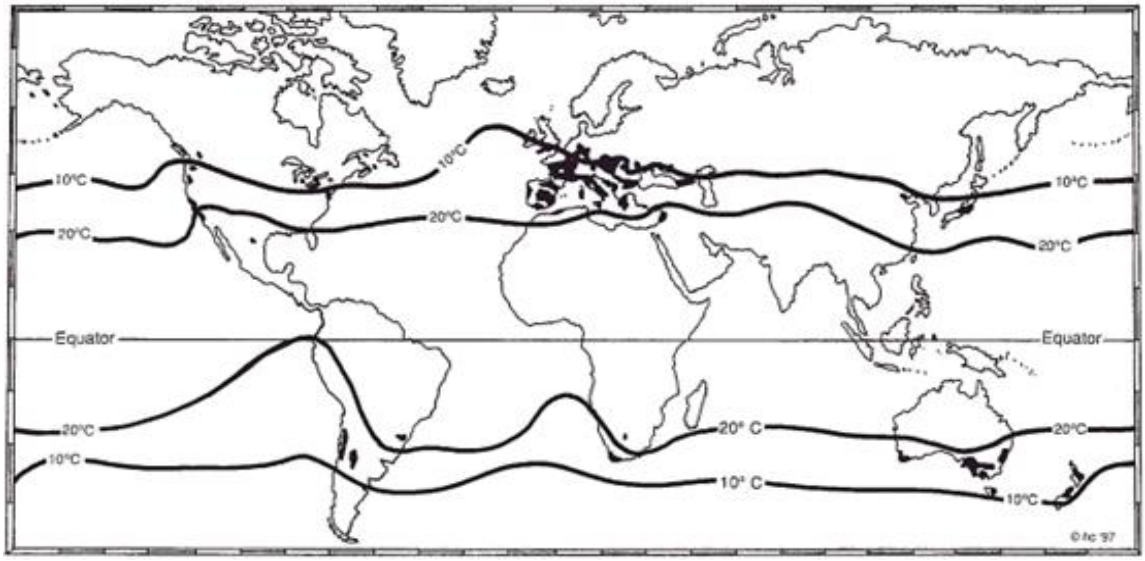

Figure 3. Association between the major viticultural region of the world, between 10 and 20°C annual isotherms (from Jackson 2008).

1.3. Systematic classification

The grapes belong to Rhamnales order, a Vitaceae/Ampelidaceae family that's divided into two subfamilies: Lecoideae and Ampelideae (Table 1).

Ampelidea subfamilty group 5 kinds of genus: *Ampelopsis*, *Cissus, Parthenocissus*, *Ampelocissus* and *Vitis*. The first four genuses are used for ornamental aims.

The *Vitis* genus include circa 40 Asiatic species and circa 30 American species as well belonging at two subgenus: *Muscadinia* and *Vitis* (Fregoni 2005) that will be described and discussed to follow.

The North American *Vitis* genus is usually employed, directly or, most of all, by hybridization in order to obtain different varieties useful to be used as rootstock for grape production *V. rupestris*, *V. riparia* and *V. berlandieri* must be mentioned regarding

rootstockings job, whereas *V. labrusca* and *V. aestivalis* are also used in hybrid fruit-bearing jobs.

In Europe, at the moment, *V. riparia* is just farmed for domestic use. In south east Asiatic countries is planted *V. amurensis* (Failla 2007).

Kingdom	**Plantae – Plants**
Underkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Magnoliopsida – Dicotyledons (Dicots)
Underclass	Rosidae
Order	Rhamnales
Family	Vitaceae
Genera	*Vitis* L.
Species	*Vitis vinifera* L.
Subspecies	*Vitis vinifera* L. subsp. *vinifera* – cultivated grape *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel) Hegi – wild grape

Table 1. Systematic classification (from Reveal 1995).

1.3.1. Subgenus *Muscadinia*

The main characteristics are summarized in points:

- The number of basic chromosomes is n=20 or 2n=40.
- The canes have prominent lenticels because of the corky layer just beneath the epiderm. Only the epiderm -- not the bark -- falls off at maturity.

- The phloem fibers of the secondary phloem are radically placed, like two uneven columns, underneath the pericycle.
- The wood is hard, without large vessels.
- There is little pith.
- There is no diaphragm interrupting the pith at the node, so the pith is continuous from one end of the cane to the other.
- The tendrils are opposite the leaves, always simple and intermittent.
- The vegetation is always glabrous or nearly glabrous.
- The clusters have relatively few berries that ripen unevenly and drop off one by one at maturity.
- The berries are pulpy, with little juice, but generally have low concentrations of sugar and are less suited for vinification.
- The seeds are navicular with an oval chalaza surrounded by radiating ridges and furrows. Seeds without ritidoma.
- The leaves are always palmate and nearly entire without lobing.
- Grafting between species of this section has not been tried because there is no practical interest in it. Grafting with species from Vitis has not been successful. The species in this section will not root from cuttings, but do root by layering.

Subgenus Muscadinia comprises: *V. munsoniana*, *V. popenoei* and *V. rotundifolia* varieties.

The first two present not real worth in agronomical field, while *V.* rotundifolia, being resisting at crittogamic diseases, as well as at phyllossera and nematodis, is used in ibridation programs combined with *V. vinifera*, in order to obtain new phyllossera-proof and nematodis-proof rootstocks.

V. rotundifolia is a typical specie spread in American and Mexicans southern coasts. (Fregoni 2005; Galet 1979; Kubitziki 2007).

1.3.2. Subgenus *Vitis*

The main characteristics are summarized in points:

- The number of basic chromosomes is n=19 or 2n=38.

- The canes have an inner corky layer, outside of which is the bark (including the pericyclic fiber, the primary and part of the secondary phloem) which may shed in strips at maturity.
- The secondary phloem has alternating tangential layers of hard and soft phloem.
- The secondary wood is soft with large vessels.
- There is a substantial amount of pith.
- A section of the shoot or cane is always elliptical and never quadrangular. There is a diaphragm which interrupts the pith at the node.
- The tendrils are opposite the leaves, two- or three-forked.
- There are woolly, bristly or special hair types on the vegetative organs.
- The clusters have numerous berries that adhere to the stem until maturity or beyond.
- The berries have a sugary and acid juice suitable for eating fresh or making juice or wine.
- The seeds are pyriform with presence of ritidoma.
- The leaves are generally palmate with five principal veins.
- All the species of this section are graftable on each other, but grafting with species from Muscadinia has been found unfeasible. The species in this section will root from cuttings.

Vitis subgenus can be divided by climate and different diffusion of the species in geographical regions (Fregoni 2005; Galet 1979; Kubitziki 2007).

1.3.2.1. Eurasian *Vitis* species

They are typical and widespread in temperate and cool-temperate climate, they originally come from Asia (*V. vinifera* too) and we can divide it in two underspecies:

- *Vitis vinifera* L. subspecies *sylvestris* (Gmelin) Hegi: wild, dyoic, and by millenniums growing spontaneously in Europe. It climbs on bush trees and it has got more resistance to diseases yhan cultivated varieties of Vitis. From female trees grape is picked up to make an acid and tannic wine.

- *V. vinifera* L. subspecies *vinifera* (for convenience purpose from here reported as *V. v. vinifera*): hermaphrodic, still now the most farmed specie in the world for his taste qualities, but extremely vulnerable to diseases. It origins from *V. v.* sylvestris by bud mutation (Fregoni 2005)

1.4. Botanical description of *Vitis species*

V. vinifera, also called Eurasiatic or European grape, because it is a typically old world species where lasts from ancient times, is a sarmentose shrub, woody and long-lived (on wild state is to consider perennial) despite intensive and specialized farming is to blame for vineyard life span drastic dropping because of losing efficiency on productivity or phytopatologic diseases (Angelini 2008).

Vitis, like other perennial woody bushes, is framed by root system (hypogeous part), cauline system (aboveground part). This last one made up herbaceous (crown) and woody (skeleton) structures.

In most of world wine-producing areas (except Chile, some areas in United States and sandy areas in Europe), *Vitis vinifera* is cultivated with the grafting of a *V. v. vinifera* cultivar combined with an American hybrid species used like rootstock, to gain strength against Phyllossera attacks that, in a notoriously way, it is a northern American native insect, one of the the most dangerous *Vitis* enemies, spread in Europe from the last XIX century quarter.

This allows *V. vinifera* to be the only epigeous part of the tree while the root system is made up hybrid varieties, obtained typically by crossbreeding *V. riparia*, *V. rupestris* and *V. berlandieri* (Failla 2007).

1.4.1. Habit

The woody part of the plant is to be articulated with, generally articuled in a main trunk, from which secondary branches. From these last ones come out buds, made up by a small branch where lateral buds and leaves find place with bunches of grape and tendrils (Fig. 4). This structure represents the tree crown. Whereas a wild grape tree gets a shruby or liana-framed morphology, sometimes monotrunched (it means with only one) sometimes politrunched, the cultivated grapes gets winegrower-wanted morphology by pruning. From a general point of view, what characterize modern grape farming way of working is low presence of intermediate woody structures between trunk and crown. Grape's traditional ways of farming feature, instead, high old wood levels because of avoiding prunning.

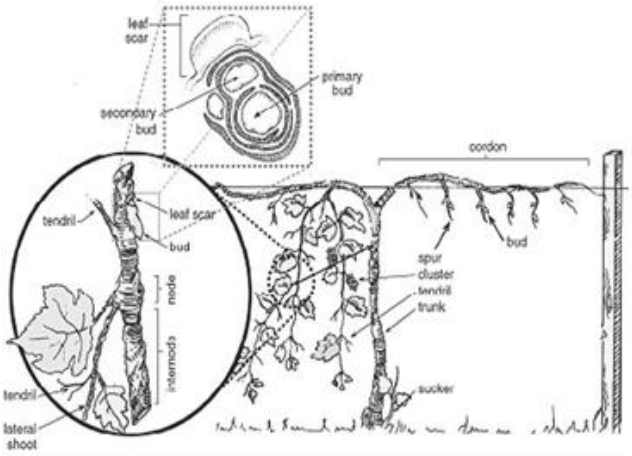

Figure 4. Domestic grapevine habitus.

From a morphologic point of view it is possible to pick out different way of farming characterized by the presence of one or more permanent branches, called cordon or cane (if long and horizontal), that grow from trunk. If they are not long and horizontal they are called spur. This grows directly from branches, related to bud from where they develop. These bud, if longs, are called vine shoots, on the other hand, if they are shorts are called spur. Branches and trunk look consist in typical external bark morphology, called rhytidome (Failla 2007).

1.4.2. Root system

Root is the shrub's organ responsible in anchoring the said shrub at the ground and providing water as well as fundamental substance to it. Into root, moreover, substances coming from epygeal area keep gathered in order to be consumed during vegetative awaking.

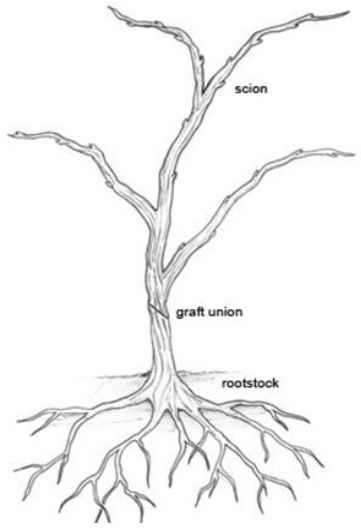

Figure 5. Root system of domestic grapevine.

Root growing from seed's embryonic rootlets (radicle) is tap root, in fact, few days after germination, all around main root start growing other more developed rootlets (crown roots) whereas radical system that grows from the base of branch's cutting shows a fibrous shape (fibrous root system), and roots are considered adventitious because junction point between radical system and trunk hasn't got, thus, collar same characteristics (Fig. 5).

In specific high air humidity conditions (lasting showers, greenhouse farming, etc.) roots can generate, under vine shoot branch node, some air roots able to reach remarkable lengths.

An adult shrub radical system can reach a considerable develop, and in farmed plants it's almost limited at one metre underground (but it can reach also 6-7 metres) and spread laterally along few metres (until 4-5 metres), dependent on ground features and thickness of plants.

Root's deepness, usually, grows with *Vitis* age. Radical system expansion depends on rootstock but also on ground, climate, cultivar grafted, the kind of culture (Leaf mass), and on farming practices as well (soil managing and above all irrigation).

The radical system habit use to be characterized by the presence of two or more different roots levels. A first level is situated along first 40-50 cm in deepness, where better conditions for mineral feeding get found. It's surface, in fact, the best area where soil present

higher chemical fertility levels and best aeration conditions, which are both vital for soil biological activity where roots grow. Other levels of radical system find themselves at a deeper area and are important. above all concerning hydric nutrition. The structure and deepness of radical system change frame along and because of stratigraphic units.

By a morphologic and functional point of view the radical system is divided into three different hierarchical orders: sustaining roots (first order), exploring roots (second order) and absorbing roots (third order). Sustaining root is life lasting, with large diameter (0,6-10 cm) and ensures mechanical stability for the tree. From sustaining roots branch exploring roots, themselves to be considered sustaining roots but with a small diameter (2-6 mm) which take on horizontal and vertical directions exploring soil in a way that follows features earlier point out. The third hierarchic level roots have short-life and around 1 mm of diameter, they grow thick during spring, from about one month after budding to one month after flowering and, sometimes, in particular conditions, afterwards grape maturation. These roots die after few weeks they are burned.

A radical system feature is the geotropical angle (genetic character transmitted to offspring in an intermediate way) obtained by comparing ideal trunk projection with real trunk state.

In a large geotropic state correspond a superficial radical system, like in the case of *V. riparia* characterized by an 80° angle; at the contrary *V. rupestris* has got a 20° angle with a much deeper radical system giving the tree lot of resistance to drought.

Indeed many others species with larger geotropical angle got more resistance (*V. berlandieri*), since resistance to drought depends also on radical system's absorbtion skills and on stomatic regulation skills as well.

The root system of cultivated grape is usually supplied by the rootstock, but in some areas (Argentina, California, Cyprus, Mexico, France, Chile, Southern Sardinia) European *Vitis* have got own roots. It depends on climatic as well as edafic features that don't fit

Phylloxera developing; in France and Southern Sardinia (Sulcis), for example, *Vitis* with own roots are cultivated on sandy grounds, where the parasite can't reproduce itself. Also in warm countries (ex. California) limited areas can be found because high temperatures ban phylloxera parasite in reaching whole reproduction cycle, while on northern zones severe weather forbid parasite's whole reproduction.

Unfortunately not yet some kind of cheap chemical product able to kill parasites avoiding pollution has been found (Fregoni 2005; Failla 2007).

1.4.3. Shoots

Young ramifications of branch are called shoots (crown's constitutive parts), they could also be called grafts, if they rise up from branches or trunk, and sucker if they come out from pedal or roots. Shoot is made up by an axis metameric. The single metamero's element features a shoot point where leaf is inserted. The space between two node or shoot points is called internode (Fig. 6).

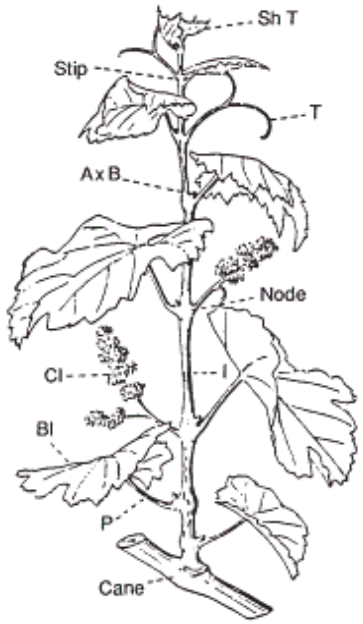

Figure 6. Vitis vinifera shoot, showing the arrangement of leaves, clusters (Cl), and tendrils (T); A x B, axillary buds; Bl, blade; I, internode; P, petiole; Sh T; Stip, stipule (from Pratt 1988).

Leaves are put singularly in each shoot node (alternate disposition). The angle which gets formed between two leaves insertion shoot node is about 180° (couplet disposition), so leaves are placed on buds in two opposed lines. This particular leaves' position is called alternate and couplet too.

First two internode of shoot are quite short, so that as result they are quite close and practically placed on the branch at the same level where shoot in located. These first two internodes are the crown of the shoot. The next internode

looks typically short with a variable length from few mm to 1 or 2 cm. Following internode got normal length (about five to ten cm).

The first crown's node hasn't got leaves whereas the second got small leaves. Sometimes these leaves are so deformed and prematurely deciduous, than they follow with normal sized leaves.

At the axil of every leaf is found a lateral bud budding directly. The shoot that grows from lateral bud is called femminella, armpity bud or axis shoot.

Lateral bud is also called ready bud. This is correct only for those growing on the shoot's newly formed stretch because of they effectively give origin shoot immediately after these last ones develop them self.Spur shoot, in this case, also take the name sillettici shoot. In the preformed bud's stretch (stem), at the contrary, lateral bud were already present like draft, at the early leaf's axil, inside hibernating bud, since summer preceding budding.

By 2^{nd}-4^{th} node shoot, following the less visible ones, stands first grape bunch opposite the leaf, otherwise you found a first tendril if bud, from which bud is grown, hadn't been able to distinguish itself. Like it will be better described afterwards, bunches and tendrils are homologous items both grown out from the top of bud.

Starting from second branch point the position of bunches and tendrils follow a precise sequence. They are spotted in an inconstant way following the sequence 1-1-0 (1=yes, 0=no). In other words, every two knots points with bunche or tendril always follow one without. Dorsoventrality is another morphologic characteristic of wine shoot. It can be picked out a dorsal side towards which leaves and spur shoots are directed, and a ventral side towards hibernating bud steer (Failla 2007).

Spur shoots (early branch) is a shoot that grows and develops in the same season time during which their buds grow on leaf's axil. Spur shoot's development starts earlier also ahead leave's stretching. Its growth is extremely variable. Through gudgeons may take form undergudgeons with capacity to bring grape towards maturation (Fregoni 2005; Failla 2007).

Towards the summer's end starts shoots lignification phenomenon “agostamento” and it consists in “turning-into-wood” shoot. Today, those shoot, are often called wine-branch. They get a darker colour and much more consistency, whereas cortical parenchyma hoards reserve substances. During cold periods the turning-into-wood process gives wine-branches necessary skills to overcome on heavy weather severity (Fregoni 2005).

1.4.4. Buds

Grape's buds are laterally situated as opposed to axis of the shoot, also inserted on node to leafs' axil. Buds are covered by shard (chips), perules as well as hairs; inside them they got one or more axil surrounded by little leaves on which it's already possible to find bunches' origins and tendril's origins too. In the *Vitis*'s genus are not distinguish fruit bud, wood bud but only mixed bud.

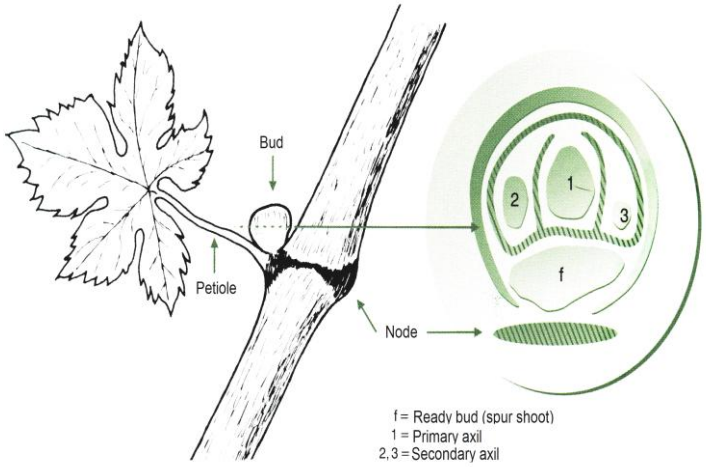

Figure 7. Ready bud (from Failla 2007).

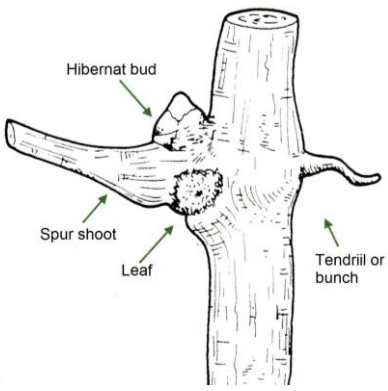

Figure 8 Hibernating bud (from Chauvet and Reyier 1979).

The bud of the grape distinguished in:

- Ready buds: they grow during spring-summer time and their development circle is less more than one month (Fregoni 2005) (Fig. 7).

- Hibernating, Dormant or Mix buds: at the bottom of the Spur shoot, in a way so close to bud to be practically beside armpit, grow hibernating bud, that are destinated to stay in a dormant condition until next year's spring. On node's preformed

traits' shoot points hibernating buds start their develop at the end of the winter, still inside the bud from which shall grow the bud on which they are inserted (Fig. 8). That happens during bud's swelling which occurs ahead bud's unfolding towards the end of winter.

In the new-formed shoot's trait, ready buds develop themselves earlier than hibernant buds. In general terms, dormancy condition is characterized by temporary buddind incapability. That, however, doesn't mean meristematic apex is inactive. In fact many dormant stages can be actually identified. Immediately after its formation bud's dormancy is defined mutual related because of its ties to principal bud and spur shoot growings. In this physiological condition bud doesn't sprout, but apex, contained in between two or three bracts called perulae, keeps in intensive activity producing up to twelve knot points with own leaves' drafts, bunches and tendrils. Budding process stops with stepping into a following dormancy phase called endodormancy.

At the bottom of hibernant bud, after three-four weeks since its development starts, and after several node points get drafted, inside perulae grow two lateral buds drafts called sub-buds, which are going to stay dormant themselves too.

The hibernating bud that hence contains perulaes, the just described structures' complex called principal bud with also its sub- bud has to be considered a compound bud. If bud also contains the bunches' drafts is to be defined a mixed bud and it is going to create a fertile shoot or a bunch bud, otherwise it will speak about woody bud from where an unfertile bud shall grow, where instead of bunches it will find only tendrils (Fregoni 2005; Failla 2007).

Sub-buds are intended to substitute the principles in the case these last ones get damaged, typically because of hibernat, thanks to their bigger strengthens. The presence of sub-buds allow trees not only to well-vegetating in case of damage caused by great chill, but also to bear since they are generally fertile, even though less than principle ones (Scienza et al. 2007).

- Latent buds: in case sub-buds wouldn’t bud during the spring following the one when they got formed, still could stay in a longer dormancy period and became latent buds. Latent buds keeps there connection to the vascular system of the shoot where it is insert, so following wood's diametrical increase. Latent buds are a growing-potential tool used by shrub in case of damaged crown or a mean managed with the aim to gain living space. From latent buds may origin some adventitious buds which have particular vegetative characteristics usually called shoots and suckers. Although these two words are sometimes used like synonymous generally shoot which grow on trunk which grow on old wood they are called suckers. It's about buds with a great potential in growing that, in nature, allow the damaged tree in recovering its crown, or to occupy new free spaces that for a reason or another became vacant among the vegetation.

In farming conditions shoots and suckers alter the tree's shape as well as the vegetal-productivity of the tree so in high figure they are usually eliminated.

The grape can't generate adventitious buds. These grow in an autonomous way on cortical tissues; they are common in many woody plants and, like latent buds, develop both vigorous suckers. In vineyards production annual pruning prompts a great imbalance between the crown and the root system in favour of this last one. This imbalance generally causes a high increase in sub-bud development that can be find out through the presence of so called double buds and crown's buds otherwise pointing to became latent buds by avoiding budding. This bust in growing give problems in recognise the real shoots' origins that come out from trunks and old wood. Lot of these buds are actually adventitious in a strict way but they come from buds from the “crown-buds made by” and suckers removed by green pruning during previous season (Failla 2007).

Bud's fertility is intended the average number of brunches produced by a single bud. Fertility is distinguished in real f. and anatomic f. and the second one is usually superior than the first one.

In the vineyard, for many reasons, the number of bunches that can be observed (real fertility) is lower than the number of potential fertility: failure in buds' budding, reduced number of brunches due to sudden chill periods or filatura.

The real fertility besides depends on pruning and nutritional conditions (when azote lacks there is no differentiation), but also by the position on vine-branch as well as the buds' number on said vine-branches. *V. vinifera*, for example, has got higher fertility in the central side of the vine-branche despite the basal zone or apex; moreover it goes down if buds' number increases on vine-branches.

In American species buds' fertility along vine-branches it's higher and constant (Fregoni 2005).

1.4.5. Leaves

Leaves are the site where very important primary physiologic processes like photosynthesis, breathing and transpiration take place. The grape leaf simple long petiolated with two stipules prematurely caducous, from deeply palmatifid to lobed and with dentate margin (Failla 2007).

At the insertion of the petiole on the limbo basal sinus with the leaf sheat 5 main venations spread called terminal or central, upper lateral and lower lateral, along the sheat and up to the apex fo the lobes. The leaf lobes are separated by the upper and lower lateral sinuses (Fig 9). Leaf shape is frequently orbicular even if it could show some polymorphism since rounded, cordate, cuneiform, reniform or more or less oblong have been observed. The basal sinus, that is an important morphological feature for a taxonomical point of view, may be variously shaped, v-shaped, u-shaped, lyrate with or without overlapping lobes outlines. The secondary venation spreads from the primary thus reaching the margin of the leaf at the apex of a teeth

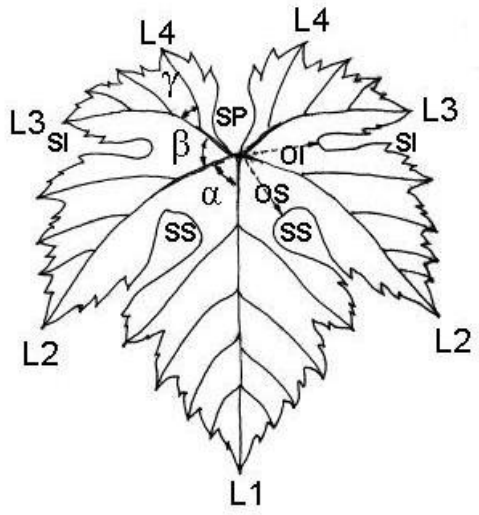

Figure 9. Leaf identify element. Nerves: mibrid (L1), upper (L2), lower (L3) and peziolare (L4). Distance or deepness of the senus: upper (OS) and lower (OI). Angle leaf: á (L1-L2), â (L2-L3), ã (L3-L4) (from Galet 1956; Galet 1991).

with an hydathode, a peculiar secretory tissue that secretes water though epidermis pores in the process so called guttation.

The epidermis of the adaxial face of the leaves is covered by a wax cuticle that is missing in the abaxial face. Abaxial epidermis, provided with stomi (approx. 200/mm^2), may be glabrous or tomentose for the presence of hair with different length and density. The hairiness of both upper and lower epiderms may deeply affect the different colour and the general aspect of the leaves (Fregoni 2005; Failla 2007).

Leaf abscission generally occurs through two points of abscission: the first is between the bud and the petiole while the second is located between the petiole and the sheat. In some varieties, the abscission occurs first at the second point, thus causing the falling of the sheat before the petiole. During fruit maturation berries in red fruited grapevines (vineyard) typically turn on reddish whereas in white fruited grapevines turn on yellowish. Moreover also leaf color may drastically change at the stage of senescence or if during summer environmental conditions becomes less ideal.

The so called “tintorie” varieties, that are characterized by coloured fruit not only on skin but also on flesh, tipically have reddish leaves during the whole growing season (Failla 2007).

The polymorphism of the leaves is used to discriminate cultivars of domestic grapevine by Organisation internationale de la vigne et du Vin protocols (OIV 2001).

1.4.6. Cirrus or tendrils

Cirrus, or tendril, is the characteristic element by which *Vitaceae* grasps tutors and climb along them. In *Vitis* tendril is forket or double forket and origins from branch point that's situated opposite to the leaf, following the development (ontogenesis) that goes on under already described rules. Like for the bunch, that's the vegetative homologous of the tendril (Fig. 10), is possible to distinguish a petiole, two main ramifications and eventually some secondary ramifications.

The tendril during its growing it matures stretching sten and ramifications either. These last ones' extremities perform slow movements following a circular or elliptical trajectory called circumnutazione movements. Those movement stopped when the ramification across mechanical obstacle. Thank a sensitivity tactile, the tendril started the spin process around at potentially support. The tendril that fails to cling to any support in general atrophied and may fall from the knot point to abscission (Failla 2007).

1.4.7. Inflorescence and flower

Figure 10. Tendril (from Watson 1992).

Grape's inflorescence is commonly referred as bunch although by a botanic point of view it isn't correct. Actually it is a complex inflorescence, definable also as a modified raceme composed of reduced shoots and flowers. Inflorescence branching is basipetal with branching details repeated at increasing levels (paracladia). The main inflorescence axis is directly divided from node shoot in a position opposite the leaf. The inflorescence part is called peduncle. Afterwards the axis forks itself with a

ramification. The first element typically consists in a tendril, usually premature deciduous, and the second element keep growing until it becomes a rachis. On the rachis are divided secondary and tertiary axis called little rachis. On winged bunches as well as on double bunches petiole's ramifications consist in inflorescence. The grapevine inflorescence is composed of a basal part, called the penduncle, two main branch and of a various number of subbranches that spreads from the main inflorescence axis and terminating in the pedicels. Flowers are arranged in groups made up of three or five – pieces-unities – called head or dichasium (Fig. 11). In its entirety a single inflorescence can be composed by few dozens up to some hundreds flowers.

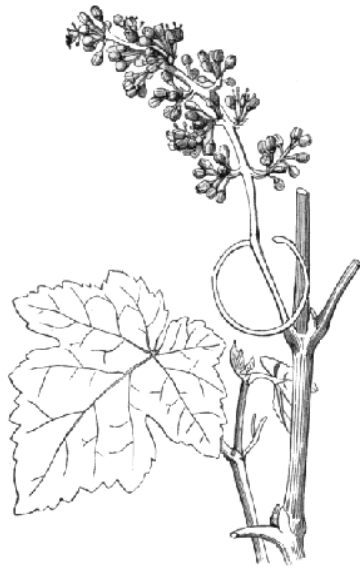

Figure 11. Inflorescence.

The grape flower is small and not showy. The flower of cultivated grape is typically hermaphrodite, pentamerous and born on a short peduncle that widen into a receptacle. Five petals are arranged during flower development in a sort of dehiscent goblet called calyptra so that the corolla is calyptrate. As the bloom starts, flowers usually do not open in a radiant corolla but their petals, greenish-colored like the most of *Vitaceae*, fall uncovering ovary and stamens. The androecium is composed of five stamens while the gynoecium is composed of a pistil with a bicarpellate, syncarpous, superior ovary and a short style. The basal part of the pistil is enlarged and by its side there are some glandular structures called nectarines. Actually these nectaries are not productive but are responsible to produce the typical (perfume) scent called “terpenico”. The anthers are born on relatively long filament (Fig. 12) while an aposepalous calyx composed of 4-5 rudimentary and very short sepals is visible at the apex of the receptacle after sepal dehiscence.

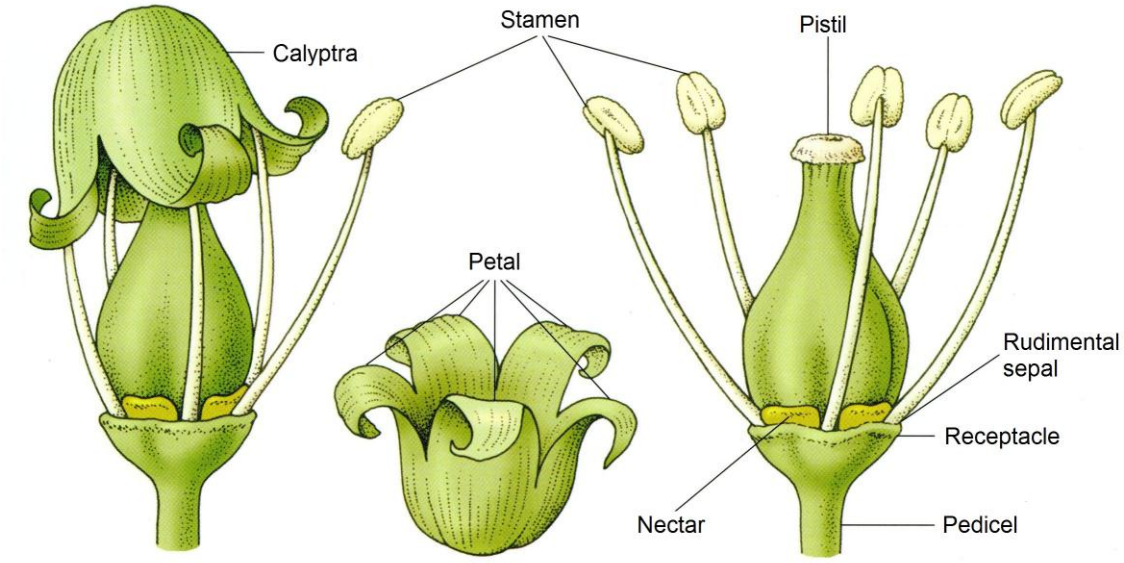

Figure 12. Hermaphodite flower of *Vitis vinifera* L subspec. *vinifera* (from Failla 2007).

The wild grapevine plant is dioecious with a unixesual flowers (in rudimentary organs) of the opposite sex; the female flower is characterized by reflex stamens and infertile pollen that does not germinate (Kimura *et al.*, 1997; Caporali *et al.*, 2003) while the male flower has an underdeveloped modified carpel (Carmona et al 2008).

The figure 13 show diagrammatic rappresentation of the variety of male, female and bisexual (hermaphroditic) flowers produced by Vitis vinifera L (Jackson 2008).

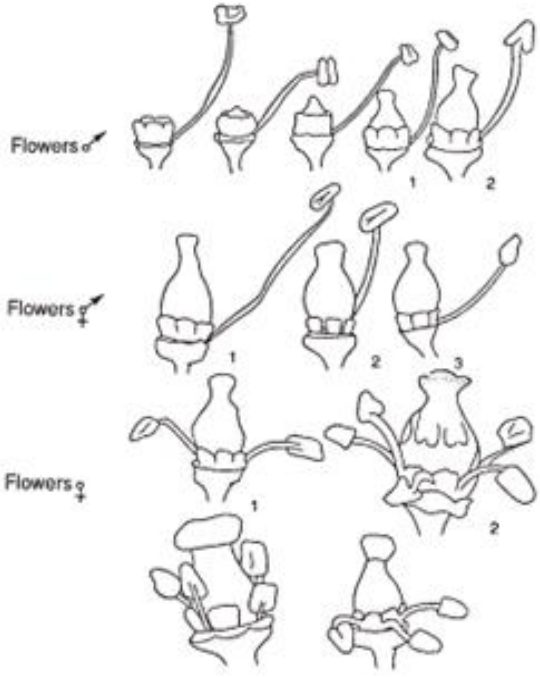

Figure 13. Flower types in wild grapevine.

When flowering takes place the petals separate along the lines of dehiscence and curve upward and outward thus freeing the anthers. During anthesis the bilocular anthers thus releasing the tricoloporate pollen in the atmosphere. Stigma receptiveness is dislpayed by the releasing of a drop of liquid which allows pollen to adhere to the stigma and to germinate into the pollen tube. The pollination process in cultivated grapevines is cleistogamous since anther dehiscence occurs while the

flowers are still wrapped by their calyptras. This feature of the grapevine flower is responsible for the attraction of pollinator insects while the pollen is not capable of long distance transport.

Every ovule contains the so called embrionic bag that represents, under a biological point of view, the female equivalent of the grain pollen. The pollen grain reaches the ovule through the tube: by the union, according to a dynamic that's far from the topic dealed by this volume, of these two gametofiti shall develop the grape's seed (Failla 2007).

1.4.8. Berry and bunch: anatomy and development

Normally impollination and fecundation process prompt the reprise of ovary's development and give this last one the possibility to become fruit. Maybe a little paradoxically, in grape like generally in all of fruit trees, the most of flowers (40-80%) don't become fruits but fall at the end of flowering (flowers abscission).When the fruit-set percentage results lower its spoken about coulure. Some flowers have got a beginning in becoming fruits and it can be noted by mean of a incipient swelling of the ovary that quickly stop with fruit drop. The percentage of flowers becoming fruit stand at 20-60% average, regarding only bunches with few flowers. Some berries start becoming fruits but stop with precocity their growth. These berries have not any seed or have rudimentary seeds (seedless berries). Berries without seeds (apyrene) develop through parthenocarpy (fruit development without seeds). In grape the parthenocarpy phenomena may show up in many ways causing the growing of berries with different features. In common bunches brands, close to normal berries so called because they got one or more seeds, it is possible to find berries with green apyrene, sweet and stenocarpy (fruits with small seeds). Green apyrene consists in the development of berries without seeds that keep in small dimension and besides they are unable to mature further. Instead, in sweet apirenia, itself too without seeds, berries accomplish maturation even though they keep small. Stenocarpic berries have got

rudimentary seeds but maturation get accomplished reaching greater dimensions than berries affected by sweet apyrene.

Sweet apyrene is a consequence of the stimulate parthenocarpy while green apyrene of the vegetable parthenocarpy. In the first the development is stimulate due to impollination of the fruit and finished because not occur the fecundation and the seed development. The stimulate of the impollination permits the berry activation and the maturation process. In the vegetable parthenocarpy instead the development of the ovary processes without the impollination of the flower and this event doesn't allows fruit maturation.

In sten ocarpy apyrene process may verify both impollination and fecondation but the seed formation early stopped and in the berry rest seed rudimental only. The seed start development bring respect as the case previous described.

Acknowledge, “acinellate” berry or with green and sweet acinellatura, may be present more or less frequently in bunch that present the upper part of normal berry. Often the acinellatura results more relevant and so we can speak of “impallinatura” of the grape.

A variety producing brunch with only apirene berry with a sweet “acinellatura” is Corinto, cultivated for raisins grape production. Sultanina grape is another variety that present great importance in the production of apyrene grape used for the making of table grape. and raisins grape too. Sultanina is a kind of grape characterized by stenospermocarpia. The great number of table grapes recently developed in genetic-improvement programs have got this kind of partenocarpyc phenomena.

Typical grape shape is the pyramidal one; it comes from basitono gradient of rachis'ramifications. When gradient is less marked bunch takes troncoconiche-cylindrical shapes; when, instead, it is more marked bunch can became wingied or compound. Like already said, both winged bunches and double bunches can derive from a bud-in flowering-differentiation of both peduncle's ramifications.

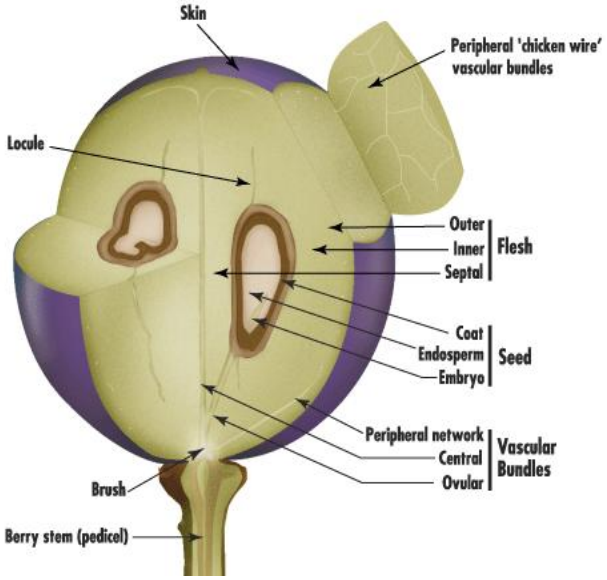

Figure 14. Structure of a ripe grape partially sectioned on the long and central axis to show internal part.

The level of compactness regarding the future grape bunch is related to the amount of peduncles and their strength (Failla 2007).

From the botanical point of view the vine fruit is a berry.

The fruit (pericarp), whose tissues are derived from the ovary, consists of the peel or dermal system (exocarp), the flesh (mesocarp) and a thin inner skin that separates the pulp from the seed lodges (endocarp) (Fig. 14).

The skin, which at maturity is between 5-20% of the fresh weight of berry, is in turn composed of an epidermis, disposed in a single layer, composed of flat cells, strongly welded together, with a thickened wall and externally covered with cuticle and epicuticolar waxes i (bloom), and a hypodermis of 11-12 layers of cells with thickened wall and strongly welded together (collenchimatic cells), mixed with cells containing, in immature berries, needle-like crystals (Rafidia) of calcium oxalate. The pulp is formed of 25-30 layers of thin-walled cells (parenchyma cells). The endocarp is of small thickness; it consists of an epidermis in a single layer and 2-3 layers of collenchimatic cells rich, in unripe berries, of quadrangular crystals (druses) of calcium oxalate (ipoden inside).

Through the peduncle, bundles conductors of the lymph penetrate the berry; they are divided into a network of dorsal or peripheral beams, which spread in the outer part of the pulp, and in a central or ventral bundle, which reaches the opposite pole of the berry reconnecting with the peripheral bundles. From the ventral beam ovular bundles also branch out to reach the seeds. When you tear from the peduncle a berry not fully mature, the central

beam is welded to it, forming the so-called brush. But when the berry is ripe, the detachment occurs at the rim of the peduncle, where it forms an abscission layer.

During the fruit set the ovary tissues resume growth. More precisely, the cells of all tissues begin to lay and to multiply, gradually assuming the characteristics described above. In particular, the cells of the epidermis strongly modify the characteristics with a marked thickening of the cuticle and the deposition of large bloom. The stomata, present on the epidermis of the ovary, much less in number than those present in the epidermis of the leaf lower (1-2 per mm2), are inactivated for the suberisation of cells below and take the appearance of pores, visible to the naked eye in non-pigmented mature berries. The cells of hypodermis assume a shape variable according to their position closer to those of the pulp from the inside. The cells of the pulp assume a spherical shape with walls increasingly thinner.

The growth of the berry, so much as in weight as the volume, is described by a double sigmoidal curve, divided into at least three stages. Phase 1, the so-called herbaceous, because the berry keeps vegetative characteristics, stage 2 or stagnation, because it the berry stops growth, and phase 3, or maturation during which the berry, profoundly alters mechanical properties and composition. In the course of the herbaceous phase, berry accumulates progressively tartaric acid and malice acid. In the exocarp also accumulate tannins (polymers of catechism and proanthocyanidins); during this phase the grape grows. After phase 1, the berry has a stagnation in growth and a reduction of biosynthetic activity, limited to the synthesis of malic acid. The duration of the synthesis can vary from a few days, in early varieties, up to 20-30 days in those later. During the stasis, the formation of grape complete with the maturity of the embryo (Failla 2007).

1.4.9. Seed: morphology and development

The grape derives from the development of the ovule; this process is triggered by the fertilization. When the development is completed, it presents a characteristic morphology and a brown colour. The seed have pear-shaped with the extremity pointed defined beak; on berry is facing the stalk. It distinguishes a dorsal, because facing the outside, where you locate a thicker area of circular shape, chalaza, which is the point where the vascular bundles were inserted in the ovary and the seed during development. From chalaza in the opposite direction to the beak departs the raphe, which runs, slightly raised, the whole ventral side. Alongside the raphe formed two depressions called dimples (Fig. 15).

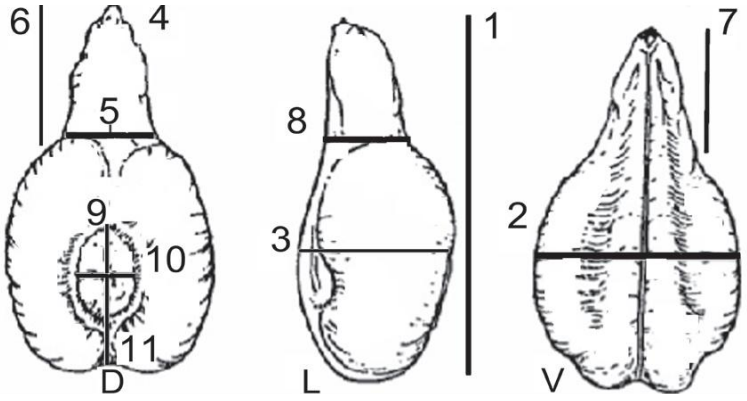

Figure 15. Seed of *Vitis vinifera* L. Dorsal side (D), side view (L) and ventral side (V). Parameters studied: total length of seed (1), maximum breadth (= total diameter *sensu* TERPÓ) (2), thickness of seed (3), breadth of the beak at the hilum (4), breadth of the beak at the seed base (5), beak length in dorsal view (6), beak length in ventral view (7), thickness of the beak at the seed base (8), total length of chalaza scutellum (9), maximum breadth (diameter) of the chalaza scutellum (10), distance from the chalaza apex to the apex of the seed (11). The index maximum breadth (diameter)/total length is also calculated for each seed (from Rivera et al. 2007).

From the anatomical point of view, the grape has two coats, a thick-walled outer (head) consists of several cell layers, and an inner end (tegmen), which derives directly from the ovary. The mechanical consistency of the grape is due to lignification of the inner layers of the outer skins. Inside the integument

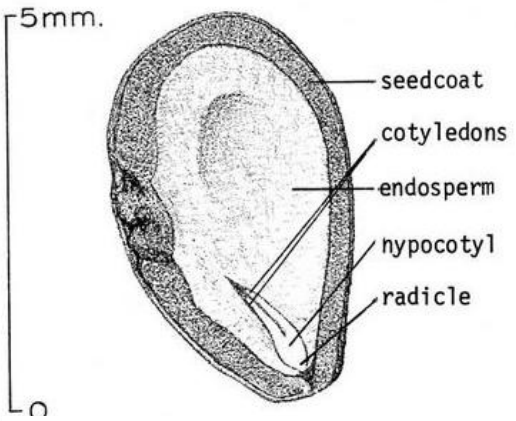

Figure 16. *Vitis vinifera* L. seed anatomy.

there is the endosperm, the tissue where the substances are stored reserves that will allow the embryo to germinate. The embryo is placed into your mouth with the radicle pointing to its tip. The endosperm is particularly rich in lipids (15-20%) mainly composed of triglycerides of oleic acid and linoleic acid, and protein content (7-10%) (Fig.16).

The grape seed completes its growth before the start of the maturation of the berry. During maturation, it undergoes further changes, improperly defined maturation. In particular, we see the colour change from yellowish to brownish. At the same time, the integument becomes more rough and hard. These changes, which will allow the seed to be more resistant to chemical and mechanical attack of the digestive tract of disseminator animals, are also related to changes in the degree of polymerization and oxidation of the tannins in the seed coat, which together become progressively less extractable and less astringent. For these reasons, the colouring and the mechanical strength of the seed have been proposed as indices of ripening grapes (Failla 2007).

Grapevine seeds are highly polymorphic and have a fundamental role in the taxonomic study within the genus Vitis L. (Rivera et al. 2007), in the distribution and domestication processes of the wild grapevine, as many archaeological discoveries suggest (This et al. 2006). The genotypical heterozygosis and the two reproduction strategy, sexual and clonally, in particular cross anemophily for *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel) Hegi and self-pollination for *Vitis vinifera* L. subsp. *vinifera*, guarantee new combinations of parental alleles and consequently phenotypic variations (This et al. 2006).

1.4.10. Reproductive system

In nature reproductive system is mixed. New individuals come out through generative way whether by seeds (ganerative way) or vegetative way, that is, thanks to one-two years old aged vine shoot able to set roots becoming autonomous if separated from mother-tree.

Whereas seeds give always genetically different individuals from mother-tree, vegetative reproduction produces individuals genetically identical with mother-tree (clonation). In nature, vine's ability to grow and spread in a vegetative way through shoots' radication allows trees to effectively extend through shoots issued at the base. It also allows tree, which like already said it's often a riparial liane, to regenerate in the case of "falling" from tutor tree because of upsettings in borders caused by riverbank overflowings. Even when vine behaves like rupestral shrub radication rappresents an effective spreading system. Seeds dispersal (dissemination) it's into nature up to birds, especially sturnus vulgaris, that, like hunters know well, its evolution came along with vine evolution with reciprocal gain: sturnus vulgaris has gotten a precious feeding source and vine gained a gorgeous effective mean of dissemination.

In farmed grape they don't apply seeds to get trees multiplication if not for genetic improvements aims. Grape's branch eradication has been always exploit through the scion technique.

In grape farming multiplication of trees related with scion technique took an absolute importance (Failla 2007).

1.5. Differences between wild and domestic grapevine

Vitis vinifera L. subsp. *sylvestris* (C.C. Gmel) Hegi (wild grapevine) is one of the most important species of this genus due to its agronomical interest in the maintenance of genetic variability and resistance of modern cultivars of *Vitis vinifera* L. (Cunha et al., 2007) which has been domesticated from wild populations of *V. v. sylvestris* (Levadoux 1956). These two species differ both in botanic morphological elements and ecologic conditions (Table 2) but main differences is reproductive strategy with wild grapevine plants being dioecious with anemophilous pollination and domesticated grapevine self-pollinating hermaphrodites.

Hermaphroditism was the crucial trait selected for by ancient farmers in order to warrant fruit production by each individual (Grassi et al., 2003).

Tabele 2. Morphological differences between wild and domestic grapevine based on Stummer 1911, Schiemann 1953, Levadoux 1956, Webb 1968, Olmo 1976, Olmo 1995, Failla et al. 1992, Mattivi et al 1993, Laguna Lumbreras 2003, Ocete et al 2004 and modified Orrù 2012

Type	Wild grapevine	Domesticated grapevine
Ecotype	Ecotype tipically mesophyte (river banks)	Mesophyte and xerophyte Ecotype
Habits	Lianous (morphology) (monocaule o policaule)	It acquires grapemaker-wanted morphology through breeding and pruning techniques
Trunk	Often branched, slender, bark separated in very long thin strips	Thick bark separates in wider and more-coherent strips
Vigorous	Shoots and sarrments with a not much vigorous and decidous posture	Shoots and sarmentos with a strongly vigorous but erect or decidous posture
Flower type	Unixesual flowers in dioecious plants	Hermaphrodite flowers
Leaves	Sexually dimorphic leaves; in female plants scarcely lobed; in male plants usually deeply three-to five-lobed (infrequently 7 lobed). Generally, the leaves are small, glabrous, dull, with entire margin, basal sinus is more or less open, petioles are short and slender. In some populations of Iberian Peninsula the dimorphism in leaves of *V. v. sylvestris* seems to follow the inverse rule, having male 'su-rounded' or scarcely 3-lobate leaves, and deeply (and wuth acute teeth) 5(7)-lobate leaves.	Absence of sexually dimorphic leaves; sometimes the leaves are large, from glabrous to tomentose, more or less glossy, and up to 7 lobed; basal sinus varies from open to close with overlapping margins. The leaves are barely entire or lobed with shallow sinuses, petiole thick, glabrous to drowny.
Fruit bunch	Small, globular to conical, irregular arranged, berry maturation not uniform in the infrutescence	Large, elongated, compact to well-fitted, berry, uniform in maturity
Berry shape	Small, round or oblate	Large and elongated
Berry colour	Berry generally deeply coloured, rarelly unpigmented	Berry yellow or in anthocyanin contaiing berries from pale rose to deeply colored colored (red, blue and blac k)
Sweet component in berry	Berry with a sweet juice.	Berry with a more or less sweet juice
Antocyanin profile	Several time with free esteri	In esterificated form (Pinot noir except)
Seeds	Small rounded or subglobose body, with a short truncated beak. Ventral face smooth and dorsal face with a well distinguished calaza. Width/length ratio (100 seeds) between 54 and 83 (Stummer, 1911) and between 64 e 83 (Schiemann, 1953)	Large, pyriform body with a rather long beak (Webb, 1968). Ventral face with evident margin and dorsal face with with a well distinguished. Width/length ratio (100 seeds) between 44 and 75 (Stummer, 1911) and between 54 e 70 (Schiemann, 1953)
Reproductive system	By seed and vegetative part (branch)	By vegetative part
Biotic stress	Resistent to disease	Sensible to disease
Vegetative period	Short	Long
Resistance to cold	High	Low

1.6. The process of domestication

Paleolitical hunter and gatherer man from mountain regions like oriental Turkey, North Syria and North-Oriental Iran knew well wild grape and he exploied it for feeding productions aims (Zohary 1996). The research and selection of particular phenotypes have been the basis of the domestication process of the wild grapevine, involving, over the years, radical changes both in the grapes biology, as well as bunch and grape dimension, and sugar content (Arroyo-García R. et al., 2006) and in their reproductive system, guaranteeing high production from every individual (Grassi et al. 2003), characteristics kept and spread through vegetative propagation agronomical technique (Zohary and Hopf 2000).

Domestication process of grape is associated with wine production, so fossil reperts refindings (seeds) and also vinification remainings refindings (tartaric acid) nearby Hajji Firuz Tepe site placed in north Zagros Mountains in Iran, datable back to 7500-8000 bc, allow to identify, in this region, the centre of grape primary domestication (McGovern 2003).

From the primary centre of domestication, thanks to cultural exchanges and sudden movements of Asiatic people, farmed grape has spread via vegetative into south-east Mediterranean areas to Palestine, Southern Lebanon and Jordan (Zohary and Spiegel-Roy 1975). Later, during the 3rd millennium B.C., domesticated grapevines appeared in the Near East, Southern Greece, Crete, Cyprus and Egypt. In the beginning of 2nd millennium B.C., domesticated grapevines were found in the Southern Balkans (Logothetis 1970; Kroll 1991). In the second half of the 2nd millennium B.C. they appeared in Southern and Northern Italy, Southern France, Spain, and Portugal in the second part of the first millennium (Levadoux 1956; Forni 1990; Hopf et al. 1991).

The history of grapevine domestication is still a matter of debate: there is no agreement on the localization of the area where grapevine was first domesticated and on the existence of secondary centres. McGovern and Olmo (1976) suggested a monocentric origin

of viticulture where domestication started from a restricted pool of wild plants. However, molecular investigations combined with archaeo-botanical and historical evidence support and testify domestication experiences in the Mediterranean area (Dedet 1980; Rivera- Núñez and Walker 1989; Grassi et al. 2003; Sefc et al. 2003).

Even though eurasian grape were already spreaded before glaciation thanks to dissemination made by migration birds, techniques and grape production have been known before local people met with Asiatic nomad people. There is a large gathering of molecular studies that prove western varieties of grape and eastern grapes are totally different from a genomic point of view. This allows to state that domestic grape has got a polycentric origin and so the existence of secondary domestication centres (Arroyo-García et al 2006). Independent domestication process (secondary) should have been helped by a huge diffusion of wild grape along all Mediterranean basin (Grassi F. et al., 2003; Snoussi H. et al., 2004).

Also the seeds were subjected to important modifications due to the domestication processes. The seeds of wild species, small, robust with a rounded outline or cordate, with short stalks and the flat ventrally were described with sharp angles and a strongly developed chalaza while those of cultivated species are large, elongated, oval or pyriform with an elongated stalk (Mangafa and Kotsakis 1996). Nevertheless, many factors determine the shape of the seeds, for instance the number of seeds in each berry, the size of the berry and its ripening (Jacquat and Martinoli 1999; Rivera et al. 2007).

1.7. Archaeological seed remain in the Mediterranean Area

In recent years there have been many archaeological excavations in the basin of the Mediterranean Sea, allowing the discovery of numerous seeds related to the genus *Vitis* L. In order of date, the wild grape seeds found in the cave of Franchthi in Greece and Ohalo II in Israel (Martinoli 2004) were dated at the Epipalaeolithic period (19.000–11.800 B.C.); those dug up in Balma Abeurar in S-France (Vaquer et al. 1986; Buxó et al. 1997) and Grotta

dell'Uzzo in S-Italy (Costantini 1981; Buxó et al. 1997) were dated at the Mesolithic period (about 10.000 B.C.); the seeds dug up in the two Turkish sites of Çayönü and Can Hasan were dated about to 7200–6500 B.C., into the pre-ceramic phase of the Neolithic period (McGovern 2004); the 47 jars, found in Abydos (Egypt) into the tomb u-j of Scorpio I, containing domestic grape seeds together to a certain amount of dried grapes with lots of stems, skins and dried pulp, were dated to the Naqada period in Upper Egypt (about 3150 B.C.), as well as other domesticated *Vitis* seeds found in 'En Besor, near Gaza, (Palestine) and Jericho in the southern Jordan Valley (McGovern 2003b). Moreover, in El Prado de Jumilla site, in Spain, a few of domestic seeds were found and dated at the Eneolithic or Chalcolithic period (3000 B.C.) (McGovern 2003a); a mixture of domestic and wild grape seeds were found in the the prenuragic and nuragic complex of Sa Osa (CW-Sardinia) archaeologic sites dated as Final Bronze Age and recent Bronze Age, a mixture of domestic and wild grape seeds were found in the archaeologic sites of San Lorenzo a Greve, in N-Italy, dated from the Early to the Middle Bronze Age (Bellini, 2008); while the seeds found in the lake of Livorno, N-Italy (Aranguren 2007; Forni 2007) and Tarquinia, C-Itlay (Delpino 2007), were dated back to the period of the Late Bronze Age.

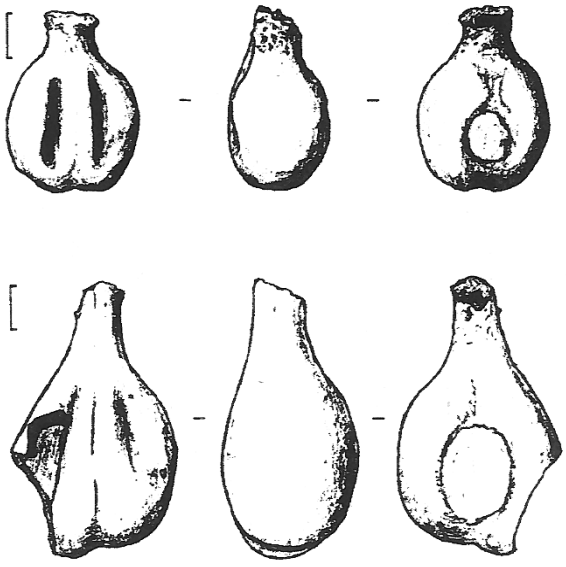

Figure 17. Iron Age pips. Upper row, pip from Duos Nuraghes. Lower row, pip from Genna Maria. Bars 1mm (from Bakels 2002).

Considering that the seeds from the prenuragic and nuragic complex of Sa Osa result to be the oldest finding of domesticated *Vitis* seeds dug up in Italy and among the most ancient remains of domesticated *Vitis* seeds found in the Mediterranean area. Other Sardinia site where was found archaeological seed belong *V. v. sylvestris* are: Duos Nuraghes

(XIV-X B.C.) (Fig. 17), Genna Maria (X-VI B.C.) (Fig. 17), Nuraghe Ortu Còmidu datable to the Punic period and Nuraghe Toscono referable to the Roman period (III B.C. - V A.D) (Bakels 2002). While off of the Island of Coltellazzo, were found same different types of seeds inside of two Phoenician amphoras (Fig. 18), referable to the Punic period (*VI-III B.C.*). have been assigned to *V. v. vinifera* (Marinval and Cassien 2001).

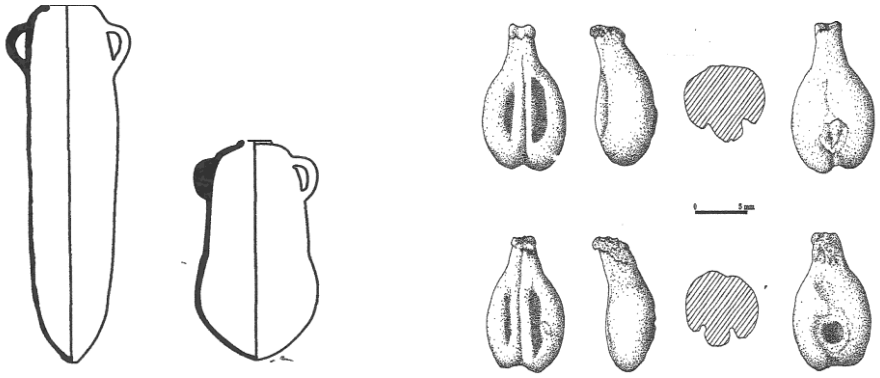

Figure 18. Different types of *Vitis* L. seeds inside of two Phoenician amphoras referable to the Punic period (from Marinval and Cassien 2001).

1.8. Aims

The aim of this work is to study the biodiversity and relathionships between wild and domestic grape to define a suitable conservation programme for wild subspecies and offer a scientific contribution to the knowledge of the *Vitis* L. genus. These results will be obtained by:

1. Investigation of dormancy and germination requirements seeds of wild grapevine to characterize the thermal niche under a Mediterranean climate.

2. Development of “Vitis L” macro to obtain the morpho-colorimetric data for each seed.

3. Devolpment of a morpho-colorimetric database and a statistical classifier able to identify and compare the wild grapevine, the cultivars of the domestic grapevine and archaeological seeds using a Linear Discriminant Analysis (LDA).

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2. Thermal niche for seed germination in the wild grapevine *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel.) Hegi

2.1. Abstract

In this study, the thermal requirements for seed dormancy release and germination of *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel.) Hegi were investigated in four Sardinian populations. Dried and fresh seeds from each population were incubated in the light at a range of temperatures (10, 15, 20, 25 and 25/10°C), without any pre-treatment and after a warm (3 months at 25°C) or a cold (3 months at 5°C) stratification. Cold stratification released physiological dormancy, while very few seeds germinated without treatments or after warm stratification. Fresh, cold-stratified seeds germinated significantly better at constant temperatures ≥ 20°C (> 80%), except for the Fluminimaggiore population, whose seeds reached ca. 60% of germination, irrespective of temperature. Germination of fresh seeds at temperatures < 20°C was negatively related to seed moisture content, highlighting a delayed development from the two high-altitude populations (Aritzo and Laconi; > 700 m a.s.l.). Although drying alone did not improve total germination, it widened the temperature range at which seeds reached high germination percentages after cold stratification. A base temperature for germination (T_b) of 9-10.5°C and a thermal time requirement ranging from ca. 60°Cd to ca. 130°Cd for maximum germination (70-90%), and from 30°Cd to 76°Cd for the 50% of germination, depending on provenance and germination temperature were identified for non-dormant cold-stratified seeds. These thermal requirements, allowed the thermal niche for seed germination to be identified and field emergence from February to May to be predicted for the investigated populations on the basis of their environmental heat sum.

2.2. Introduction

The *Vitis* L. genus consists of ca. 65 inter-fertile species growing almost exclusively in the Northern Hemisphere (This et al., 2006). *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel.) Hegi (wild grapevine) is one of the most important taxa of this genus due to its contribution of genetic variability and resistance to modern cultivars of the domestic grapevine *V. v. vinifera* (Levadoux 1956; Cunha et al., 2007). Plants of these two taxa differ in their reproductive strategy and habit. Wild grapevine plants are dioecious, with a dimorphic foliage (i.e. the leaves of male plants being more deeply lobed), berries that are ca. 6 mm long, ellipsoid, bluish-black and acidic and seeds that are subglobose, with a short truncated beak. In contrast, domesticated grapevine is a self-pollinating hermaphrodite, with 6-22 mm long berries, that are ellipsoid to globose, green, yellow, red or purplish-black and sweet; seeds are pyriform, with a rather long beak (Webb, 1968). *V. v. sylvestris* reached the Mediterranean probably from the Caucasic area (Grassi et al., 2006) and is now distributed from North Africa up to Central Europe (Arnold, 1998). It grows from 0 to 1000 m a.s.l., along riverbanks, on screes (colluvial sites) of hilly humid slopes and occasionally on coastal sheers and beaches (Ocete et al., 2008). The present distribution of the wild grapevine is highly fragmented in disjointed micro- or meta-populations constituted by few individuals (Terral et al., 2010), due both to an increased human impact on lowland areas (especially on the the western part of the Mediterranean Basin) and new pests that came from North America about 150 years ago (Grassi et al., 2006).

Temperature is the main environmental factor governing seed germination in moist soil, determining both the fraction of seeds in a population which germinate and the rate at which they emerge. The rate of germination usually increases linearly with temperature, at least in a well-defined range and the accumulated temperature for germination (also called “thermal time”), above the base temperature for germination (at which the rate of germination is zero), is a constant which can be used to compare germination in seed lots of different

species, genotypes, climates and localities (e.g García-Huidobro et al., 1982). Quantification of seed responses to environmental stimuli has been applied to the dependence of germination rate on constant temperatures via probit transformation and co-plotting of germination against thermal (or log-thermal) time, e.g. for crop species (García-Huidobro et al., 1982; Covell et al., 1986) and trees (Pritchard and Manger, 1990; Pritchard et al., 1996).

Intra-specific variation in seed dormancy release and germination can be attributed to differences in genetic characteristics and environmental conditions during seed development and maturation and those in the post-dispersal environment (Donohue, 2005; Giménez-Benavides et al., 2005; Mondoni et al., 2008). Base temperatures for germination varied with seed lot provenance in *Quercus robur* L. (Pritchard et Manger, 1990) and seeds of *Aesculus hippocastanum* L. from more southerly locations in Europe, e.g. Greece, were able to germinate at cooler temperatures, due to a lower base temperature for germination, than seedlots from further north, i.e. France, Poland and England (Daws et al., 2004).

Freshly extracted seeds of *Vitis* ssp. usually show a high proportion of physiologically dormant seeds which can require considerable periods of cold stratification before they will germinate (e.g. Singh 1961; Ellis *et al.*, 1983; Ellis *et al.*, 1985; Conner, 2008; Wang et al., 2011). Subsequent efficient seed germination occurs at 20-30°C, suggests that emergence will occur naturally in the spring after winter chilling (Baskin and Baskin, 1998). Wang et al. (2009) modelled the effect of stratification on dormancy release in Beichun grape (a cross-breed of *V. v. vinifera* and *V. amurensis* Rupr.) seeds and found that dormancy was consistently released with prolonged stratification time at temperatures < 15°C, while stratification at 25°C induced secondary dormancy. Alternatively, dry after-ripening can break dormancy in grape seeds (Ellis et al., 1985). For example, in *V. amurensis* seeds dormancy was released by 90 days at low seed moisture and 25°C (Wang et al., 2011). However, to our knowledge, no information is available on thermal niche for seed dormancy release and germination in *V. v. sylvestris.*

Sardinia is the second-largest island in the Mediterranean Sea. Its isolation and high geological diversity have created a wide range of habitats, especially on its mountain massifs, where there are conditions of ecological insularity (Médail and Quézel, 1997). During the quaternary glaciations, the Island constituted a climatic refuge area for *V. v. sylvestris* (Grassi et al., 2008) which is still present in numerous, well-preserved, large populations (Lovicu et al., 2009). Sardinian populations range from 60 to 800 m a.s.l. on different substrates (mainly paleozoic), except for limestones. The isolation of these populations in natural localities located far away both from urban areas and from vineyards, indicates the low probability that intraspecific hybridization with *V. v. vinifera* cultivars has occurred (Zecca et al., 2010).

The aims of this work were (1) to characterize seed dormancy and germination requirements of this poor investigated species and (2) determine its thermal niche for germination under a Mediterranean climate, following a thermal time approach. The implications of the findings in the context of the opportunity for this rare taxon to naturally regenerate in the wild are also discussed.

2.3. Materials and Methods

2.3.1. Seed lot details

Berries of *V. v. sylvestris*. were collected in the 2009-2010 period from six different localities belonging to four populations at the time of natural dispersal (Tables 1 and 2). Immediately after collection, seeds were separated from the fleshy fruits by squashing through sieves with a tepid water wash, then spread in a thin layer to dry. Seeds collected in 2009 were stored in a dry room at 15% R.H. and 15°C for 15 months. At the end of the drying period the moisture content (mc% w.b.) was calculated gravimetrically after 17 h at 103°C (ISTA, 2006), based on three replicates of 50 seeds each (Table 2). Seeds collected in 2010,

were not dried and the weight and moisture content were calculated immediately after cleaning (Table 2).

Table 1. Seed lot details of the investigated populations of *V. v. sylvestris*.

Population code	Region	Locality	Mean coordinates (Datum WGS84)	Substrate (Carmignani *et al.*, 2001)	Altitudinal range (m a.s.l.)	Habitat
SU1	Sulcis	Gutturu Mannu - Assemini (CA)	N 39° 10' E 08° 55'	Granites	86-114	Riparian woods with *Alnus glutinosa* (L.) Gaertn.
SU2	Sulcis	Medau truba manna – Siliqua (CA)	N 39° 12' E 08° 46'	Metamorphytes	119-183	
IG1/2	Iglesiente	Rio Antas – Fluminimaggiore (CI)	N 39° 24' E 08° 28'	Metamorphytes	130-270	Riparian woods with *Alnus glutinosa* (L.) Gaertn. –Mantle shrubs with *Rubus ulmifolius* Schott
SA1	Sarcidano	Cuccuru – Laconi (OR)	N 39° 52' E 09° 05'	Granites	715-718	Mantle shrubs with *Rubus ulmifolius* Schott - Deciduous woods with *Quercus sp pl.*
SA2	Sarcidano	Mitz'eurgia carbone – Laconi (OR)	N 39° 52' E 09° 05'	Granites / Travertines	622-756	Mantle shrubs with *Rubus ulmifolius* Schott - Deciduous woods with *Quercus sp pl.*
BA1/2	Barbagia di Belvì	Errist'aulu - Aritzo (NU)	N 39° 55' E 09° 15'	Granites	710-780	Deciduous woods with *Quercus sp pl.*

Table 2. Variation in weight and moisture contents for *V. v. sylvestris* seed lots. Data are the mean of three replicates of 50 seeds each. Values with the same letter are not significantly different (P > 0.05) by One-way ANOVA followed by *post hoc* Fisher LSD test.

Population code	Collection Date (day / month / year)	Fresh seed weight (mg; mean ± SD)	Moisture content (%; mean ± SD)	Dry seed weight (mg; mean ± SD)
Dry seed lots				
SU1	20/09/09	-	5.42 ± 0.05 a	26.05 ± 0.44 a
IG1	03/10/09	-	5.52 ± 0.04 ab	25.83 ± 0.22 a
SA1	09/10/09	-	5.66 ± 0.09 c	24.46 ± 0.21 b
BA1	25/10/09	-	5.60 ± 0.04 bc	24.67 ± 0.16 b
			P < 0.01	P < 0.001
Fresh seed lots				
SU2	09/10/10	32.35 ± 0.40 a	19.70 ± 0.09 a	25.98 ± 0.35 a
IG2	18/09/10	32.68 ± 1.55 a	17.48 ± 0.37 b	18.99 ± 0.89 b
SA2	28/10/10	35.43 ± 1.18 b	28.80 ± 0.44 c	25.23 ± 0.82 a
BA2	23/10/10	36.95 ± 0.35 b	22.31 ± 0.26 d	28.71 ± 0.18 c
		P < 0.001	P < 0.001	P < 0.001

2.3.2. Germination test

Three replicates of 20 seeds each were sown on the surface of 1% agar water (with the ventral face of the seeds turned towards the germination substrate) in 90-mm diameter plastic Petri dishes and incubated in the light (12 h light ⁄ 12 h dark) for a minimum of 45 days and a maximum of 233 days at a range of germination temperatures (10, 15, 20, 25 and 25⁄10°C). Further replicates were given a warm (3 months at 25°C) or a cold (3 months at 5°C) stratification, before these temperature treatments. In the alternating temperature regime, the 12-h light period coincided with the warm period. Germination was defined as visible radicle emergence to ≥ 1 mm and germinated seeds were scored three times at week. At the end of the germination tests, when no additional germination had occurred for 2 weeks, a cut-test was carried out to determine the viability of the remaining seeds. Soft, mouldy seeds were considered to be unviable.

2.3.3. Data analysis

Seed germination response to temperature for non-dormant seeds has been described using a thermal time approach (García-Huidobro et al., 1982). In this model, seeds accumulate units of thermal time (°Cd) to germinate for a percentile g of the population, when subjected to temperatures, T, above a base temperature for germination (T_b). T_b is generally assumed to be constant within a population, but below an optimum temperature T_o. When the thermal time accumulated has reached the critical value (θ_{Tg}) for a percentile g of the population, germination occurs in the time t_g. Thus, the thermal time required for a fraction g of the population can be described as:

$$\theta_{Tg} = (T_g - T_b)t_g$$

For the various seed lots of *V. v. sylvestris* the mean base temperature for germination (T_b) was determined for dry seed lots from 2009 (see table 2), but after 3 months cold moist stratification to remove dormancy. T_b, in the sub-optimal range, was averaged from the *x*-intercepts of the linear regressions between constant temperature (10-25°C) and germination rate ($1/t_g$) for each g percentile (from 10% to 90%) of the populations (Ellis *et al.*, 1986; Pritchard and Manger, 1990), according to the following equation (García-Huidobro *et al.*, 1982):

$$1/t_g = (T_g - T_b) / \theta_{Tg}$$

Thus, to describe the form of cumulative germination response of seeds the following equation was used (Covell et al., 1986):

$$\text{probit}\,(g) = K + \log \theta_{Tg} / \sigma$$

where K is an intercept constant when thermal time (θ_{Tg}) is zero and σ is the standard deviation of the response to $\log\theta_{Tg}$ (i.e. the reciprocal of the slope), and represents the sensitivity of the population to $\log\theta_{Tg}$ (Covell et al., 1986). Thus, the flatter the slope of the fitted line, the greater the variation in response to thermal time between individual seeds (Daws et al., 2004). On a plot of probit(g) against $\log(\theta_{Tg})$, the median thermal time required for seed germination of the population (g = 50%; θ_{T50}) corresponds to the thermal time when probit(g) = 5 (Daws et al., 2004).

Environmental heat sum was calculated starting from March (i.e. after the winter cold stratification period), for each population (see Table 1) as follows:

$$\text{Heat sum} = \Sigma\,(T_m - T_b)\,t_m$$

where T_b is the base temperature for germination, T_m is the average monthly temperature of the month *m* and t_m is the number of days of the month *m*, until reaching the critical value of thermal time (θ_{Tg}) in °Cd for the maximum percentile of germination. Linear regressions were fitted using SigmaPlot 2002 for Windows version 8.0 (SPSS, Chicago, IL, USA).

Climatic data (historical series of monthly averages of temperatures and rainfall from nearby climatic stations) were achieved for each population site (weather stations of Desulo, Fluminimaggiore, Laconi and Capoterra, for Barbagia, Iglesiente, Sarcidano and Sulcis, respectively), from Regione Autonoma della Sardegna (http://www.regione.sardegna.it/j/v/25?s=131338&v=2&c=5650&t=1).

One-Way ANOVA was carried out on seed moisture content of fresh seed lots, to test for differences among populations. Final germination percentages and base temperature values were analysed with One-way ANOVA, subsequently, a *post hoc* Fisher least significant difference test (LSD) was conducted. For these analyses R v. 2.11.1 (R Development Core Team, 2010) was used.

2.4. Results

Freshly collected seeds of *V. v. sylvestris* germinated in a significant way only after a cold stratification period (C), while a very few seeds germinated in the control (0) or after warm stratification (W) for all the investigated seedlots (Fig. 1). After 3 months cold stratification, seeds from all lots germinated significantly better ($P < 0.05$) at constant temperatures ≥ 20°C (> 80%), than at 10 and 15°C (< 40%) except for IG2, whose seeds germinated with percentages ranging from ca. 40% (15°C) to 60% (25°C), without significant differences among temperatures (Fig. 1). Fresh, cold stratified seeds reached their maximum germination when incubated in the alternating temperature regime (25/10°C), with final

percentages of 98 ± 3% for BA2 and SU2 seed lots and 95 ± 5% for IG2 and SA2 seed lots. This condition was the only temperature regime that allowed some seed germination (ca. 25%) for the SA2 seed lot without any pretreatment (Fig. 1).

Dried seeds showed a similar trend, with seeds germinating in a significant way only after cold pretreatment, particularly when moved to constant temperatures ≥ 20°C and at 25/10°C in all seedlots (Fig. 1). However, dried seeds germinated better (> 40%), although still significantly lower ($P < 0.05$), than the fresh ones also at temperatures lower than 20°C (i.e. at 10 and 15°C), except for BA1 with ca. 20% of germination at 10°C (Fig. 1). Few dried seeds germinated also without any pretreatment and after warm stratification; in particular, seeds from IG1 germinated to ca. 40% at 25/10°C without any pretreatment, while SU1 seeds reached ca. 10% at almost all temperatures in the control and after warm stratification pretreatment (Fig. 1).

The great majority (ca. 90%) of non-germinated seeds from the tests on both freshly used and pre-dried seeds appeared to be viable at the end of the experiments when checked by the cut-test.

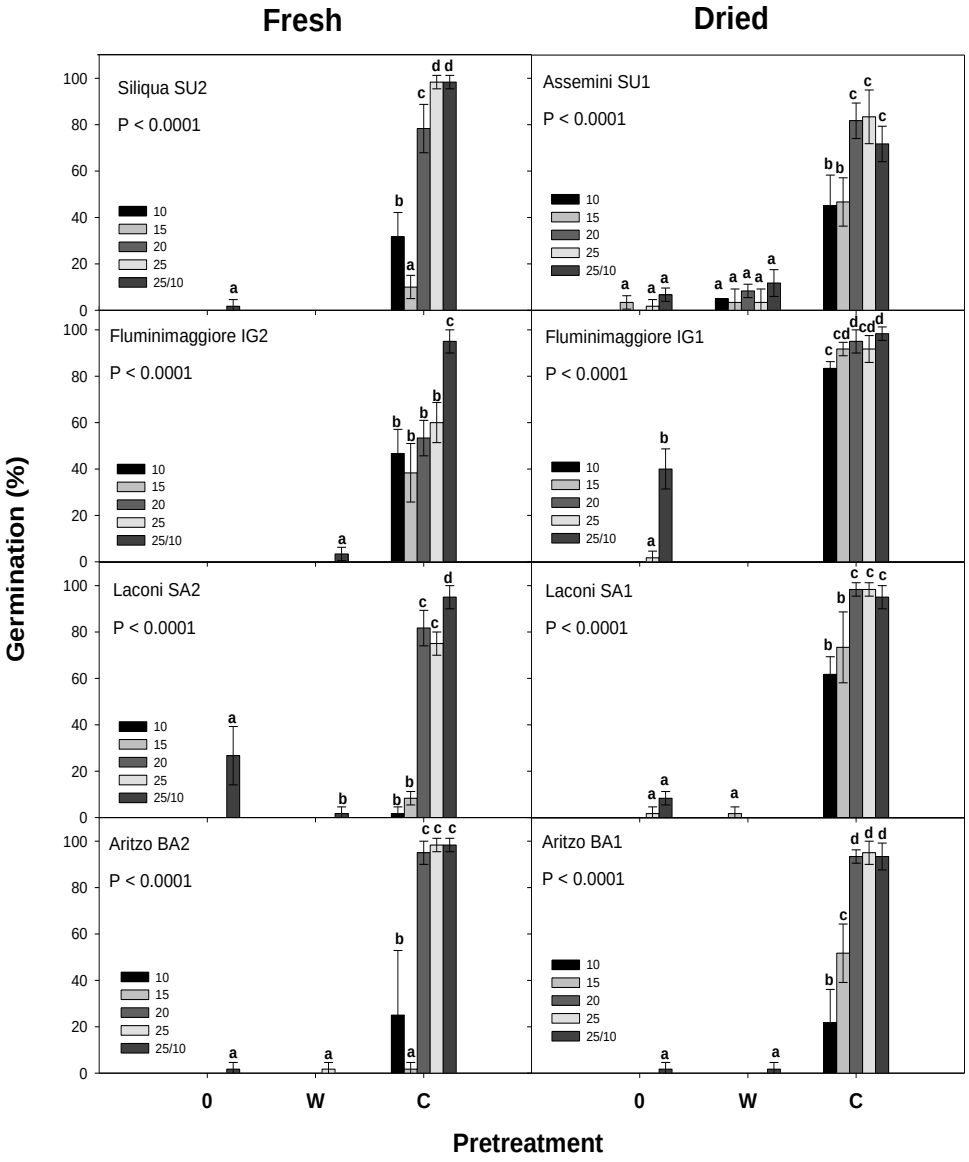

Figure 1. Final germination percentages (mean ± 1 SD) of dry and fresh seeds for the four investigated populations at different temperatures for each pretreatment: 0 = control, W = warm stratification (25°C for 3 months) and C = cold stratification (5°C for 3 months). Data are the mean of three replicates. For each seedlot One-way ANOVA, followed by *post hoc* Fisher's LSD test, was carried out; bars with the same letters are not different at P > 0.05.

Seed moisture content of freshly collected seeds varied significantly ($P < 0.001$) among seedlots from 17.5 ± 0.4 to 28.8 ± 0.4% for IG2 and SA2, respectively (Table 2). At constant temperatures ≥ 20°C and at 25/10°C, final germination percentages of cold stratified fresh seeds were indifferent to their initial seed moisture content (linear regression $P > 0.05$; data not shown). However, at cold temperatures (10 and 15°C) these values were negatively related to the seed moisture content of each seedlot ($P < 0.001$; $R^2 = 0.41$). According to the

linear regression, germination of pre-chilled *V. v. sylvestris* seeds at < 20°C is predicted to be completely inhibited in material harvested at moisture contents (i.e. not pre-dried) above 29.2% moisture content (Fig. 2).

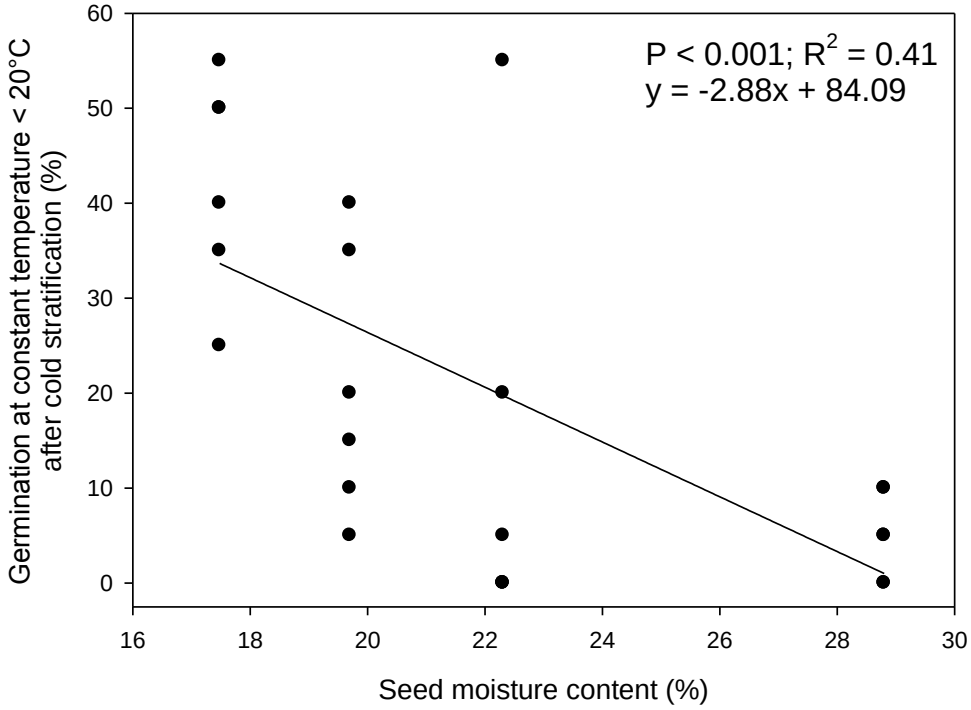

Figure 2. Relationship between seed moisture content (%) and final germination at 10 and 15°C after cold stratification (3 months at 5°C) for fresh seeds of the four investigated populations. Each point is one replicate of 20 seeds.

From the linear regressions of the relationship between constant temperatures and germination rate of dried cold stratified seeds ($1/t_g$) for each 10^{th} percentile (g) from 10% to 90% germination, it was possible to estimate the mean base temperature for germination T_b for each seedlot (Table 3). These values ranged from 9.0 ± 1.1°C of IG1 to 11.3 ± 0.7 of BA1 seed lots, being statistically different by One-way ANOVA (Table 3). Post hoc Fisher's LSD test highlighted that this difference was determined by the T_b value of IG1, as it was the only one value which differed at $P < 0.05$ from the others (Table 3). Germination percentages also increased with the tested temperatures (Fig. 1). Therefore optimal temperature for germination T_o for seeds of this species may be assumed as ≥ 25°C. It was not possible to calculate base temperatures for germination for cold stratified seeds belonging to freshly collected seed lots

due to a lower final germination percentage achieved at 15°C in relation to 10°C in three seed lots (see Fig. 1).

Table 3. Base temperatures for germination (T_b) for different percentiles and overall population means, calculated after cold stratification (3 months at 5°C) and incubation at constant temperatures (10-25°C). For percentiles for which regression lines had a P > 0.05, T_b values were not calculated. Statistical differences among seed lots were anlysed by One-way ANOVA followed by *post hoc* Fisher's LSD test; values with the same letters are not significantly different at P > 0.05.

Seed population	T_b (°C) for proportions of the seed population									T_b (°C)
	10%	20%	30%	40%	50%	60%	70%	80%	90%	Mean ± SD
SU1	11.7	10.9	10.6	10.0	10.5	8.9	-	-	-	10.4 ± 0.9 [a]
IG1	10.6	9.9	9.3	8.6	8.0	7.8	7.9	8.6	10.5	9.0 ± 1.1 [b]
SA1	11.4	10.9	10.5	10.1	9.9	10.4	9.8	10.1	11.7	10.5 ± 0.6 [a]
BA1	11.5	11.2	11.0	12.5	11.4	10.2	-	-	-	11.3 ± 0.7 [a]
										P < 0.0001

Figure 3 shows the relationship between log-thermal time and germination expressed in probits for the four investigated *V. v. sylvestris* populations. Probit germination of seeds showed similar thermal time requirements (θ) for BA1, IG1 and SA1 populations. In these populations, maximum germination (90%) occurred by 58°Cd (at 25°C) to 74°Cd (at 15°C), at 66°Cd (25°C) to 84°Cd (10°C) and from 67°Cd (25°C) to 138°Cd (10°C), for BA1, IG1 and SA1, respectively. Seeds belonging to SU1 population generally needed higher θ values; from 95°Cd (15°C) to 113°Cd (25°C) to reach a lower maximum germination (70%). Thermal units needed to reach 50% of germination (θ_{T50}) where also calculated, with values ranging from ca. 30°Cd to ca. 65°Cd for BA1, IG1 and SA1 populations, respectively, whereas SU1 needed ca. 76°Cd (Fig. 3). All seedlots had similar dose dependencies, with decreasing standard deviations of the population responses to thermal time (σ values) from high to low temperatures (Fig. 3). However, seeds of SU1 population had greater σ values (from log 1.39 to log 2.71°Cd) than the other seed lots (log 0.60 – log 0.81°Cd, log 0.82 – log 1.39°Cd and log 0.74 – log 1.40°Cd for BA1, IG1 and SA1, respectively; Fig. 3).

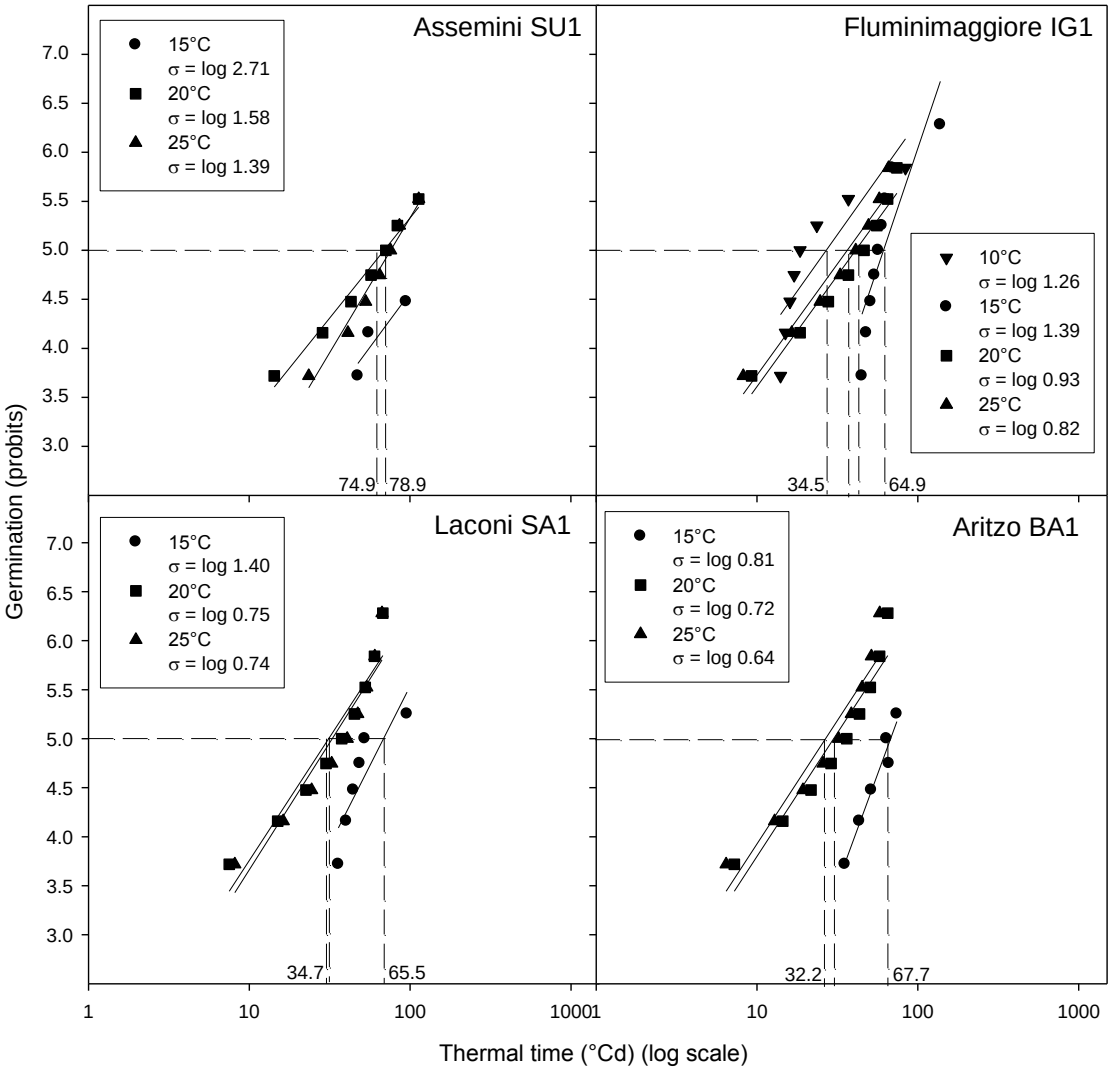

Figure 3. Germination probability of dried seeds as a function of thermal time requirement (log scale). Thermal times were calculated from germination time courses at 10, 15, 20 and 25°C, assuming base temperatures for germination of 11.3, 9.0, 10.5 and 10.4°C for BA1, IG1, SA1 and SU1 seed lots, respectively. Points are the mean of three replicates.

Available climatic data allowed the calculation of the heat sum (Σ°Cd) that non dormant seeds accumulate in each investigated population after winter, when mean temperatures (T_m) rise above the base temperatures for germination T_b (Fig. 4). Although all four populations showed a bi-seasonal Mediterranean trend they had relevant differences on their mean monthly temperatures (T_m), according to their altitudes (see Table 1). These varied from minimum values of 5.9°C, 10.2°C, 8.7°C and 10.1°C for BA1, IG1, SA1 and SU1, respectively (January; Fig.4) to maximum values ranging from 22.9°C to 27.4°C (August; Fig. 4). Due to these differences, seeds from SA1 and BA1 should start to accumulate thermal

units (°Cd) on March and April, respectively, when environmental temperatures (T_m) exceed base temperatures for germination (T_b). In contrast, the higher mean temperatures (and for IG1 also a lower T_b) should allow seeds to accumulate thermal units as early as January and February for IG1 and SU1 populations, respectively (Fig. 4). Consequently, differences were detected also on the time to accumulate their critical thermal times for germination (θ). Dried non dormant seeds belonging to BA1 seed lot needed 74°Cd to reach 60% germination when incubated at constant 15°C (see Fig. 3). According to the climatic data, seeds would reach this value in May (Fig. 4) when the mean temperature (T_m) is 14.8°C. Similarly, seeds from SA1 are expected to reach 96°Cd in April, allowing 60% of germination at 15°C, as the mean temperature (T_m) is 13.2°C (Fig. 4).

Seeds from the two lowest populations (IG1 and SU1) showed a biphasic seed germination with seeds germinating over two or three months (Fig. 4). Seeds from IG1 are predicted to start germinating from February when mean temperature (T_m) is 10.5°C (coherent with a θ value of 37°Cd for achieving 70% of germination at 10°C; Fig. 3) and complete germination in March when a mean temperature (T_m) of 12.6°C allows the remnant non germinated seeds to reach a θ value (69°Cd) that corresponds to 80% germination at 15°C (see Fig. 3). Seeds from SU1 germinate from March (T_m: 13.41°C), with a first step corresponding to a θ value of 95°Cd, allowing 30% of seed germination at 15°C (see Fig. 3) and a second flush of emergence in May (T_m: 19.9°C) corresponding to a θ value of 113°Cd, allowing 70% of seed germination at 20°C (see Fig. 3).

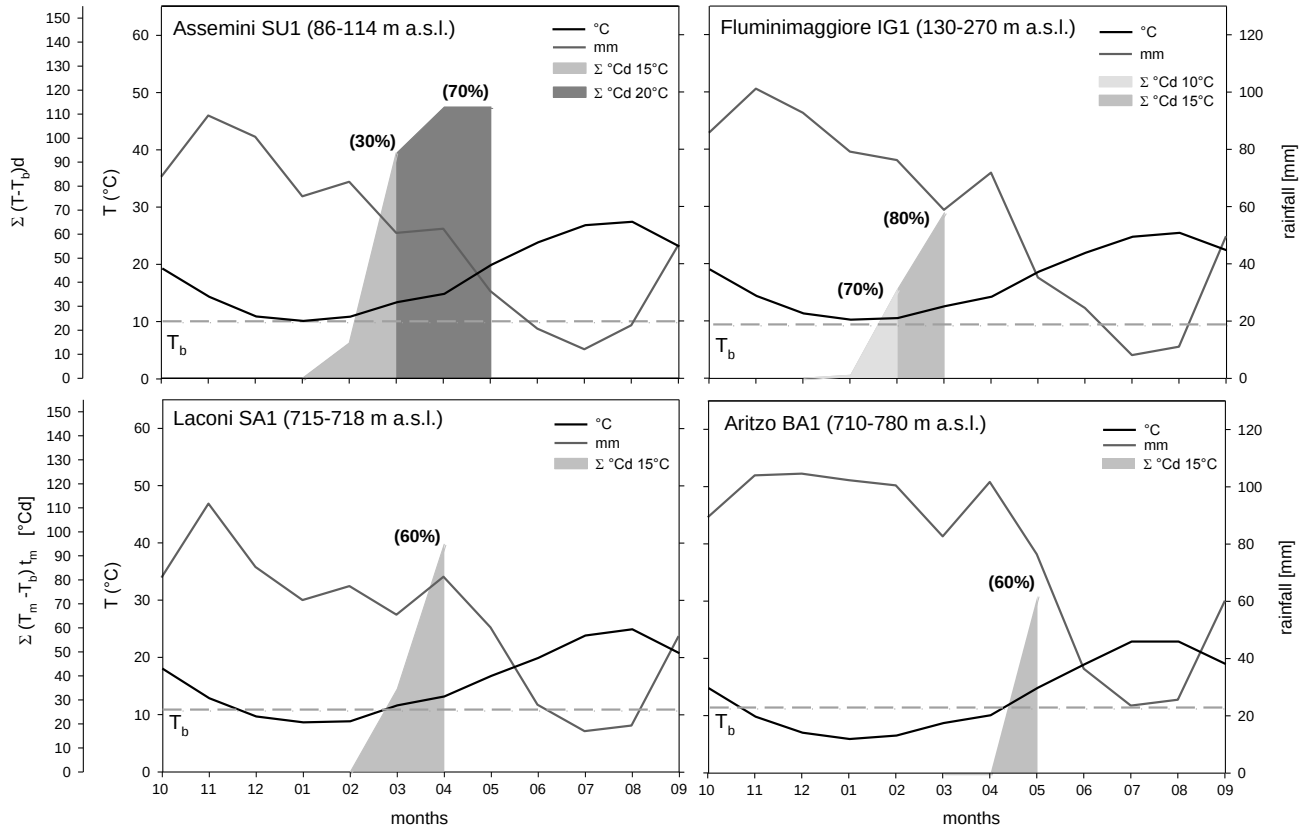

Figure 4. Environmental heat sum for dried non dormant seed calculated for each population as the sum of mean monthly temperature – base temperature for germination (T_m - T_b) per the number of days (t_m) until reaching the critical amount of °Cd (θ) for germination at 10, 15 and 20°C. Base temperatures for germination are assumed to be 11.3, 9.0, 10.5 and 10.4°C for BA1, IG1, SA1 and SU1 seed lots, respectively (dashed lines). Annual trends of temperature and rainfall are also reported for each population, starting from the month of natural dispersal (10 = October).

2.5. Discussion

2.5.1. Seed dormancy

Freshly harvested seeds of *V. v. sylvestris* from all the investigated populations did not germinate, or reached very low germination, when incubated without any pre-treatment. Thus, they are dormant, as already detected for other species of this genus (e.g. Singh 1961; Ellis *et al.*, 1983; Ellis *et al.*, 1985; Conner, 2008; Wang et al., 2011). Warm stratification *prior* to the germination test did not enhance germination, whereas cold stratification completely released dormancy. Drying had a positive effect on germination of *V. v. sylvestris*, widening the temperature range at which seeds reached high germination percentages after cold stratification. Drying before or after stratification markedly promoted the effect of 5°C stratification on seed dormancy release of different varieties of *V. amurensis* (Wang et al.,

2011), suggesting that a combination of dry after-ripening and moist cold stratification may be a common pattern of dormancy breaking for species of this genus, probably mimicking the alternance under natural conditions of moist and cold seasons (i.e. winter) with warm and dry ones (i.e. summer).

Fresh cold-stratified seeds of *V. v. sylvestris*, germinated at high temperatures in three of the four investigated populations. Following the dormancy classification system (Baskin and Baskin, 2004), seeds of *V. v. sylvestris* show a "Type 4 non-deep physiological dormancy". Such seeds gain the ability to germinate exclusively at high temperatures only after the application of a dormancy breaking treatment (Baskin and Baskin, 2004). There is limited knowledge of seeds with this type of dormancy and it has been reported only for a temperate deciduous forest shrub (*Callicarpa americana* L.; Baskin and Baskin, 2004). However, seeds from one population (IG 1/2) germinated also at colder temperatures (< 20°C) after cold stratification. Thus, the ability of excised embryos to produce normal seedlings and the effect of dry after-ripening on shorthening the lenght of cold stratification should be investigated in order to verify an "intermediate level" (*sensu* Baskin and Baskin, 2004) of physiological dormancy for seeds of this species. In addition, Forbis et al. (2002) reported that members of the Vitaceae have a small embryo to seed ratio (0.036 ± 0.004), leading to morphological dormancy in seeds of this family. Therefore, embryo growth studies during germination should be carried out to establish whether there is a morphological component to the seed dormancy of this species (i.e. morphophysiological dormancy).

2.5.2. Seed development

Environmental conditions, before and after seed dispersal, may affect seed development and germination (Thompsett and Pritchard, 1993; Donohue, 2005). Seeds of *V. v. vinifera* are reported to be orthodox (Royal Botanic Gardens Kew, 2008) and *V. v. sylvestris* seeds, considering the high germination percentages achieved at low moisture content (< 6%), are clearly highly desiccation tolerant. In orthodox seeds, continued deposition of storage materials within seeds during their development results in a decrease in percentage seed moisture content at maturation (Ellis et al., 1987; Welbaum and Bradford, 1989). Thus, the significantly different levels of fresh seed weight and moisture content in freshly collected seeds detected for the investigated populations likely reflect different levels of seed development. Seeds from the two high-altitude populations (SA2 and BA1/2, 700-800 m a.s.l.), with higher moisture contents, were slightly immature, as confirmed by the low germination percentages achieved at temperatures < 20°C and the positive effect of drying on

their germination. Also in *Aesculus hippocastanum*, seeds from cold regions were less developed at the time of natural seed shed due to a shortened developmental period modulated by lower air temperatures (Daws et al., 2004).

2.5.3. Thermal requirements for germination of non dormant seeds

The base temperature for germination (T_b) in non-dormant (i.e dried cold-stratified) seeds of *V. v. sylvestris* ranged from 9 to 11°C depending on the provenance. To our knowledge this is the first report of T_b for a member of the Vitaceae. T_b of not stratified (i.e. dormant) *Aesculus hippocastanum* seeds (English population) was found to be close to 25°C, varying slightly between collection years and decreased to 8°C after a stratification of 101 days at 6°C (Pritchard et al., 1999). Considering that no seeds of *V. v. sylvestris* germinated without treatment at the tested constant temperatures, a $T_b > 25$°C may be supposed for dormant seeds of the investigated populations. However, this remains to be confirmed by incubating seeds without stratification at warmer temperatures (i.e. up to 40°C). The detected T_b values are consistent with the current circum-Mediterranean distribution (Arnold, 1998) and the altitudinal range (0-1,000 m a.s.l.; Ocete et al., 2008) of this species, preventing seedling establishment in northern and higher-altitude regions, where spring temperatures are too low to facilitate seed germination. Seeds of European species adapted to temperate climates showed lower T_b (i.e *Quercus robur* or *Castanea sativa* with 0.8-2.4°C and 1.4°C, respectively; Pritchard and Manger, 1990), while Trudgill et al. (2000) found that T_b values of 31 wild species and four coltivated species growing in the UK varied from –1.8 to 12.0°C, according to the species, with highest values reached by the arctic-alpine *Dryas octopetala* L.

All the conditions tested in this study were in the sub-optimal range and, therefore, optimal temperature for germination of non-dormant seeds of *V. v. sylvestris* may be assumed as ≥ 25°C. Germination at high temperatures (20-30°C) is a common pattern in vines (Baskin and Baskin, 1998) and in particular in *Vitis* species (Ellis et al., 1985). The optimal germination temperature was 30°C for stratified seeds of several varieties of *V. amuriensis* (Wang et al., 2011) and Conner (2008) reported that stratified seeds of *V. rotundifolia* Michx. should be germinated in an environment where daytime temperatures reach this level.

The linear response between seed germination and temperature in the sub-optimal range identified a thermal time requirement ranging from ca. 60°Cd to ca. 130°Cd for maximum germination (70-90%), and from 30°Cd to 76°Cd for 50% of germination (θ_{T50}), depending on provenance and temperature of germination. Thermal time requirements may vary considerably between species, even among species with similar T_b (Trudgill et al., 2000).

For example, seeds of *Aesculus hippocastanum* needed a thermal time of ca. 100°Cd for 80% germination (Steadman and Pritchard, 1996) while this value increased to 400°Cd and 1000°Cd for seeds of *Quercus robur* and *Castanea sativa*, respectively (Pritchard and Manger, 1990).

2.5.4. Thermal niche for seed germination

The thermal time requirements identified in this study together with the available climatic data, allowed the thermal niche for germination to be identified and field emergence to be predicted for non-dormant seeds of each investigated population, on the basis of their environmental heat sums. In the laboratory experiments winter conditions were simulated by 90 days of cold stratification at 5°C. Climatic data available for the investigated populations showed a mean temperature from December to February (winter) of 9.0, 10.7, 9.1 and 6.5°C for SU1, IG1, SA1 and BA1, respectively. Although all these values were higher than the tested stratification temperature (except for BA1), they were lower than 15°C, the temperature threshold below which stratification consistently released dormancy of Beichun grape seeds (Wang et al., 2009). The two highest populations (SA1 and BA1) showed very similar values of T_b, and thermal times (log θ) allowing germination at 15°C in March and April for SA1 and BA1, respectively. In contrast, seeds from IG1 with lower T_b and thermal time values could anticipate germination to February, when mean the temperature is close to 10°C. In all these populations, seeds can germinate before the start of summer so that the seedlings enter the dry summer with well-developed root and shoot systems. The seedling growth period, i.e. from seed germination to the onset of the summer, increases as the altitude decreases from 1 month for BA1 to 2 months for IG1. This shows an adaptation of the germination time to the local microclimate. In contrast, seeds from the lowest population (SU1), due to their high T_b and high log θ values and to the greater variation in response to thermal time (σ values), could not germinate at temperatures < 20°C, before the start of the summer drought in May. These thermal requirements and the subsequent different times for germination limit species distribution in Sardinia along riverbanks and colluvial sites of hilly humid slopes where the species is found (Ocete et al., 2008). Plants at the lowest population (SU1, 86-114 m a.s.l.), characterized by more than four months of summer drought, were found exclusively within the riparian woods with *Alnus glutinosa*, where watercourses do not dry up in summer. At higher altitudes, some plants grow also in the mantle shrubs with *Rubus ulmifolius* (IG1/2, 130-270 m a.s.l.), whereas in the two highest populations, where precipitations are higher and summer drought shortened to a maximum of three months, plants grow only in habitats where

soils are not moistened during summer, such as the mantle shrubs and deciduous woods with *Quercus* sp. pl. at Laconi SA1 (622-756 m a.s.l.) and the deciduous woods at BA1/2 (715-718 m a.s.l.). Finally, while seeds from SU1 can germinate at 20°C even without drying, allowing field emergence the first spring after dispersal, seeds from the other three populations need to overcome one summer and dry, before cold stratification during winter may activate germination at cool temperature (< 20°C), suggesting a field emergence on the second spring after seed dispersal.

2.6. Conclusions

Fresh seeds of *V. v. sylvestris* of the four investigated Sardinian populations were physiologically dormant at dispersal. Cold stratification released seed dormancy, suggesting spring emergence by this species. Cold environments led to slightly immature seeds at the time of seed yeld for the two high-altitude (> 700 m a.s.l.) populations. The base temperature for germination (ca. 9-10.5°C) and the thermal time requirements determined in this study, allowed the thermal niche for seed germination to be identified and field emergence to be predicted on the basis of environmental heat sum. This approach confirmed a germination time ranging from February to May for the investigated populations, although this germination pattern should be confirmed by field germination experiments. The detected high T_b and optimum germination temperature prevent seedling establishment in northern and higher-altitude regions. At the same time, the germination phenology of this species limits the species distribution, in Sardinia (and in the Mediterranean region more in general), along riverbanks and colluvial sites of hilly humid slopes (Ocete et al., 2008), where seedlings can grow even during the dry summer conditions, suggesting a phenotypic adaptation to different microclimates. The reported data characterizes seed dormancy and thermal niche for germination of *V. v. sylvestris*, provides new insights on the seed biology and ecology of this rare taxon, a wild crop relative whose conservation is also of high agricultural and economic interest, contributing to the genetic variability and resistance of *V. v. vinifera*.

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3. Pips Image Analysis to support Cultivar Identification of *Vitis vinifera* L.

3.1. Abstract

Identification of cropped cultivars, as well as wild relatives of any world spread agronomic crops, is a big challenge both for economical implications and for the safeguard of special products against fraudulent mystifications. This is more true for products that have achieved the European trademark attribution, in which the used raw material play a key role.

Vitis vinifera L. is one of the most worldwide hedonistic crops with an huge multitude of trade name, in which the fundamental characteristic is the grape variety, the land of cultivation and the processing.

With this aim, we have tried to develop a system of statistical identification, based on morpho-colorimetric features measured using image analysis techniques, able to identify and classify some of the grapevine varieties of a living collection, developed and maintained in Sardinia island by Agricultural Research Agency of Sardinia (AGRIS), that moreover preserves *ex situ* the autochthonous grapevine varieties, described for Sardinia.

Pips images were acquired at the Germplasm Bank of Sardinia (BG-SAR) using a flatbed scanner, and successively analysed at the Stazione Consorziale Sperimentale di Granicoltura per la Sicilia (SSG) using a specifically developed macro based on the image analysis software KS 400.

The morpho-colorimetric data and elliptic Fourier descriptors obtained, were elaborated by Linear Discriminant Analysis to build a statistical classifier able to identify the cultivars and landraces through pip traits.

Some attempts were done to compare the cropped varieties with some wild populations of *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel) Hegi of Sardinia island and some archaeological remains of pips founded in Sardinia too.

3.2. Introduction

Sardinia, the second largest island in the Mediterranean basin, represents an interesting Italian region that thank to the spatial isolation harboured and protected the genetic richness of grapevine accessions limiting the opportunities of contamination from out-coming material (De Mattia et al., 2007). The island is characterized by the contemporary presences of numerous populations of *Vitis vinifera* and for a big number of indigenous autochthonous grapevine varieties (about 210) that seems to characterize and differentiate the regional viticulture (Lovicu, 2007).

Considering that *Vitis vinifera* L. is one of the most worldwide hedonistic crops with an huge multitude of trade name, in which the fundamental characteristic is the grape variety, the land of cultivation and the processing, and taking into account also the great implications that special products, as those with European trademark attributions, have in the economy of a land, the aim of this work was develop a system of statistical identification, based on morpho-colorimetric features measured using image analysis techniques, able to identify and classify some of the grapevine varieties and landraces of a living collection, developed and maintained in Sardinia by the Agricultural Research Agency of Sardinia (AGRIS), moreover to compare the cropped varieties with some wild populations of *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel) Hegi of Sardinia island and some archaeological remains of pips founded in Sardinia too.

The work is in progress and here are presented in detail the macro developed to obtain the morphologic and colorimetric data for each sample.

3.3. Main heading

3.3.1. Materials and Methods

Pips images were acquired at the Germplasm Bank of Sardinia (BG-SAR), according to Bacchetta et al. (2008), by a flatbed scanner (Epson GT15000), a resolution of 400 dpi and a scanning area not exceeding 2800×2800 pixels was adopted.

The individual pips were arranged on the scanner transparent flat, isolated from each other and they were also covered using two boxes, one dressed with opaque black paper and another one with white paper, to avoid interferences of environmental light. In this way, for each pip sample, two images were captured, one with black background and another one with white background, to contrast the brightest and the darkest regions of the pips.

Additionally, the standard reference image Kodak Q60 was periodically acquired to carry out a Look Up Table (LUT) useful to standardize the scanner, in order to allow the correct measurement of colorimetric data.

Using KS-400 V. 3.0 (Carl Zeiss, Vision, Oberkochen, Germany) image analysis system and its libraries, a specific macro, called *Vitis*.mcr, was developed in KLic program language, proprietary of Carl Zeiss Vision, in the Image Analysis Laboratory of the Stazione Sperimentale di Granicoltura per la Sicilia (SSG), to achieve the measurements of 33 morpho-colorimetric features of seeds (Table 1). Moreover, SHAPE ver.1.3 (Iwata and Ukai, 2002), a freely distributed software program, was used to delineate the shape of the two dimensional contours of the pips projections, using the Elliptic Fourier Descriptors (EFDs), originally proposed by Kuhl and Giardina (1982). SHAPE ver.1.3 is composed by four little dedicated programs for processing digital images: ChainCoder, Chc2Nef, PrinComp and PrinPrint. With the aid of this software package, the user can easily analyze shapes of objects without specific knowledge about the procedures involved in the method.

This program calculates a series of normalized coefficients a1an, b1bn, c1cn, d1dn, where the n value is the maximum harmonic number, that can be processed and used as descriptive variables of an object shape.

The obtained 33 morpho-colorimetric data and 80 EFDs, were elaborated with the software package SPSS (SPSS release 15, SPSS Inc. 2006) applying the stepwise Linear Discriminant Analysis (LDA) method, to build a statistical classifier able to identify the landraces through pip traits (Bacchetta et al., 2008).

Table 1. List of morphometric and colorimetric measured features on pips

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

3.4. Results

The macro *Vitis*.mcr works on the basis of the two sample images with different background colour, from here defined "original images". At the beginning they were standardized applying a Look-up Table (LUT) obtained following the Shahin and Symons (2003) protocol, as suggested by Venora et al. (2009). Afterwards, both original images were contrasted to optimize the visualization of the pips, and than segmented applying an RGB threshold method, to obtain binary images. A cleaning procedure of scrapping of singulated pixels and filling of internal holes, was than executed, obtaining two segmented images of partial Vitis pips (Fig. 1a and Fig.1b). Applying an "OR" Boolean operation, the two binary images were combined to achieve an unique segmented image in which the whole projection profile of the pips appear (Fig.1c).

A control image was created combining one of the two original images with the binary image obtained, and than displayed as a check point, allowing to the user to do little corrections editing the binary image.

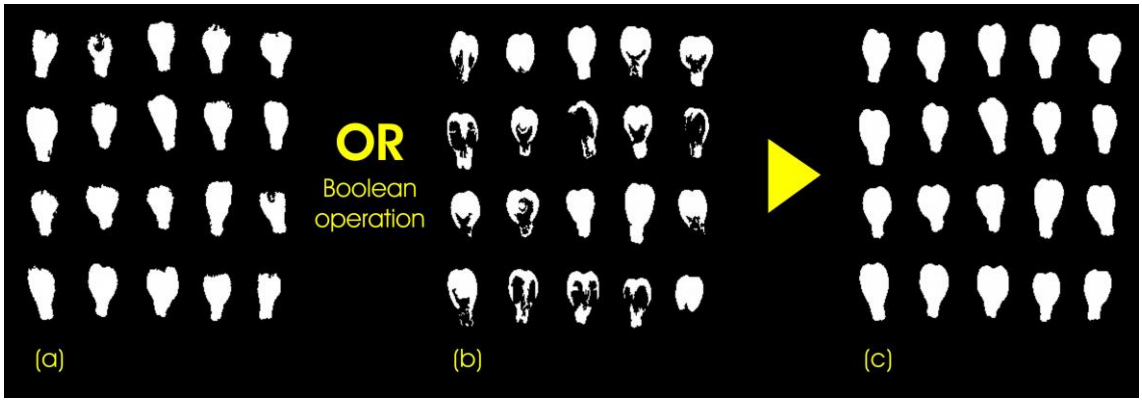

Figure 1. Boolean combination of two binary images. a) Segmented image of the black background original image; b) Segmented image of the white background original image; c) Combined binary image.

Applying a LUT, included in the libraries of the KS400 software, for the conversion of the original image colour model from RGB to HLS, a new image was created to measure hue, lightness and saturation values of each pips also for HLS model.

After having set the frame properties of the image to measure, a pre-built measure file was loaded. In it all the measurement information are included, as the selected parameters, the measurement conditions and the dimensional scale for the geometric calibration.

During the measurement procedure, the macro allows to select the pips that has to be measured or deselect those that has not to be measured for any reason. The measurement phase is composed by three parts: the first in which the RGB channels and the densitometric parameters were measured, the second in which the HLS channels were measured and the third in which all the morphometric features were measured.

Finally, a database with the measure features values was displayed, and some of the processed images were saved.

The developed macro will be applied to analyze the images of pips accessions of 300 cropped variety and landraces of Vitis vinifera, and 20 populations of *Vitis sylvestris* of 1, 2 or 3 harvest years. For each sample, 3 images of 100 pips were acquired, achieving a total of about 2000 images.

Moreover, images of a few archaeological pips, founded inside a Phoenician-Punic amphora (IV-III B.C.) on the Coltellazzo (Pula) island seabed, close to Cagliari (S-Sardinia), were acquired too.

3.5. Conclusions

The results obtained in previews similar works on cropped (Venora et al., 2007; 2009) and wild species (Bacchetta et al., 2008; Mattana et al., 2008; Grillo et al. 2010), allow to

presume that also for the genus *Vitis* the multivariate statistical techniques, based on morpho-colorimetric parameters, will allow to implement statistical classifiers able to discriminate among different groups.

The expected results will consist to draw phyletic relationships between Sardinian main cropped variety, landraces, and the founded archaeological pips too.

Moreover, considering the opportunity to dispose, shortly, of molecular data relating to the studied samples, the next step will lie in doing an accurate evaluation of the classification model implemented using exclusively morphologic and colorimetric features, with the purpose of once again, how image analysis techniques can be considered a needful tool on qualitative and quantitative evaluations.

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4. Morphological characterisation of *Vitis vinifera* L. seeds by image analysis and comparison with archaeological remains.

4.1. Abstract

In archaeobotanical studies, the taxonomic classification of diaspores has usually been done by simple morphological observation and visual comparison with *ex situ* collections of seeds, although the use of biometric indices has often proved to be a powerful approach in the taxonomic studies of the genus *Vitis* as well as for the species attribution of archaeological remains. Using image analysis techniques, seeds from two Sardinian archaeological sites, the pre-Nuragic and Nuragic complex of Sa Osa in central-western Sardinia, attested as the oldest Sardinian archaeological site with remains of *Vitis* seeds, and the Isola di Coltellazzo in southwest Sardinia, were selected and characterized on the basis of morphological features and Elliptic Fourier Descriptors. Moreover, seeds of five modern populations of *V. vinifera* ssp. *sylvestris* collected from southwest Sardinia, and the seeds of 41 cultivars of *V. vinifera* ssp. *vinifera* mainly from southern and central-western Sardinia, were also analysed by computer vision, and the obtained data were used to implement a database of biometric parameters and to compare the unknown archaeological seeds with the characterized recent seeds, using Linear Discriminant Analysis. The similarity of the archaeological seeds to *V. vinifera* ssp. *vinifera* cultivars rather than to *V. vinifera* ssp. *sylvestris* populations could allow to state that, between the Middle and Final Bronze Age, varieties very close to modern *V. vinifera* ssp. *vinifera* were already being used to produce wine and/or to preserved for foodstuffs. Moreover, the better matching of the archaeological seeds to white grapes rather than black grape cultivars, could indicate the origins of the traditional cultivation of white grapes in these regions of Sardinia.

4.2. Introduction

Grape pips are highly polymorphic and have a fundamental role for the taxonomic study within the genus *Vitis* L. (Rivera et al. 2007), for the distribution and domestication processes of the wild grapevine, as many archaeological discoveries suggest (This et al. 2006). The genotypical heterozygosis and the two reproduction strategies, sexual and clonally, in particular cross pollination by wind for *Vitis vinifera* L. ssp. *sylvestris* (C.C. Gmel) Hegi and self-pollination for *Vitis vinifera* L. ssp. *vinifera*, guarantee new combinations of parental alleles and consequently phenotypic variations (This et al. 2006).

The search for and selection of particular phenotypes have been the basis of the domestication process of the wild grapevine, involving, over the years, radical changes both in the biology of grapes, as well as bunch and grape dimensions, and sugar content (Arroyo-García et al. 2006), and in their reproductive system, guaranteeing high production from every individual (Grassi et al. 2003). Also, the seeds were subjected to important modifications due to the domestication processes. The seeds of wild species, small, robust and with a rounded outline or cordate, with short stalks and a flat ventral side have with sharp angles and a strongly developed chalaza, while those of cultivated species are large, elongated, oval or pyriform with an elongated stalk (Mangafa and Kotsakis 1996). Nevertheless, many factors determine the shape of the seeds, for instance the number of seeds in each grape, the size of the grape and its ripening (Jacquat and Martinoli 1999; Rivera et al. 2007).

In many archaeobotanical studies, the taxonomic attribution of diaspores has been done by simple morphological observation and comparison with ex situ collections of the seeds. For example, the seeds found in the Roman site of Pamplona in northern Spain, dated back to A.D. 100-300 have been identified as *V. v. vinifera* on the basis of optical stereomicroscopy observation (Peña-Chocarro and Zapata-Peña 1996). The seeds found during the palaeobotanical and sedimentological studies in the open-cast lignite mines of Hambach in western Germany, and datable to the late Pliocene were identified as V. sylvestris on the basis of visual observations (Heumann and Litt 2002), as well as the single example from the archaeological excavations carried out in Monte Trabocchetto northern Italy, referable to the early Iron Age (Arobba et al. 2003). Also, the Vitis seeds from the archaeological sites of the river Struma in southwest Bulgaria, dated to the early Neolithic, early-middle Neolithic and late Bronze Age, were identified by visual comparison (Popova and Marinova 2007), as well as those from the late Neolithic and Eneolithic or Copper Age site of Hocevaria in Slovenia, and were identified as *V. v. sylvestris* (Jeraj et al. 2009).

In Sardinia numerous seeds have been recovered at archaeological sites, with various degrees of preservation, with fragmentation and/or carbonization of the teguments. At the Duos Nuraghes site two different kinds of seeds, identified as *Vitis*, were found. Some of them have been described as squat types, with short stalks, typical of *V. v. sylvestris* and datable to the late Bronze Age (1400-1000 B.C.), while some others showed similarities with those found near the Genna Maria site in central Sardinia and referable to the Iron Age (1000-600 B.C.) (Bakels 2002). Moreover, many more seeds of *Vitis* are known from Sardinia, for example from the Nuraghe Ortu Còmidu, in the central south of the island and from the Nuraghe Toscono in central Sardinia, both dating to the Punic period, others dating to the Roman period (300 B.C.-A.D. 500) (Bakels 2002). On the occasion of all these archaeological finds, empirical methods were used to identify the seeds, making a comparison or quantitative evaluation difficult if not impossible.

During a recent archaeological excavation in April 2008 close to S'Arrieddu and the village of Cabras in provincia di Oristano, rich deposits of the pre-Nuragic and Nuragic settlement of Sa Osa were recovered (Usai 2011). Within the site, a structure (Shaft N) was identified and excavated in sandstone sediments to a maximum depth of 4.35 m (Serreli 2011). The shaft contained, in different stratigraphic units, numerous organic materials including wood, coal, cork, seeds (mostly of *Vitis*), animal remains and pottery helpful for dating it to the final and recent Bronze Age (Usai 2011; Sanna 2011). The *Vitis* seeds were studied using a binocular microscope, highlighting the prevalence of shapes of cultivated varieties (Lovicu et al. 2011).

The use of biometric indices for seed studies has often proved to be of great importance in the understanding of the domestication processes, in taxonomic studies of modern *Vitis*, as well as for the classification of archaeological remains (Rivera et al. 2007). Mangafa and Kotsakis (1996) used 22 biometric variables and four different algebraic formulae to identify the seeds of *Vitis* found at the prehistoric sites of Dikili Tash and Toumba Thessaloniki in Greece, as belonging to wild or cultivated species. Their formulae, just for their potentials, have subsequently been used for the study of the seeds of *Vitis* found at Petra in Jordan, and dated back to 200 B.C.-A.D. 500, allowing recognition of many seeds of wild plants and a certain amount from crops. The data were partially confirmed by the calculation of Stummer's index (Jacquat and Martinoli 1999). The ratio between stalk length and pip length, used to study five varieties of grapevine (Chasselas, Pinot Noir, Rèze, Amigne and White Humagne), gave different results compared with the Mangafa and Kotsakis formulae, showing that the "sylvestris" type pip morphology could be associated not only with wild

grapevines but also to archaic varieties. Consequently, the seed found in Petra could belong to a cultivated grapevine bearing fruits of wild type. This hypothesis seems to be validated by the existence of such varieties not only in Europe but elsewhere (Jacquat and Martinoli 1999).

In southwestern Sardinia, near the Isola di Coltellazzo, were found various seeds and charred woody materials in different states of preservation inside two Phoenician amphorae, referable to the Punic period (600-300 B.C.). Some of these *Vitis* seeds had an ovoid shape, a well evident and lengthened beak and the chalaza situated in the upper part of the seed. According to the morphological features and to the biometric indexes related to the ratio between pip length and pip width, the seeds were assigned to *V. v. vinifera* (Marinval and Cassien 2001).

Another study on the identification and grouping of *Vitis* seeds on the basis of biometric features, was carried out on 142 different types of grape, including five taxa of *Vitis*, 92 cultivars of *V. v. vinifera*, 12 feral or wild populations and hybrid rootstock cultivars, measuring 11 morphometric variables by an electronic caliper. The obtained data were elaborated using cluster analysis, placing feral or wild populations and related cultivars in their respective clusters, but missing a cluster of wild European grapevine (Rivera et al. 2007).

In a recent paper, Terral et al. (2010) discussed the potential of morphometric analysis to compare well-preserved archaeological seeds, found in southern France and dated back to 100 B.C., with some European modern cultivars and wild individuals, using the Elliptic Fourier Descriptors (EFDs) method. Also, Gong et al. (2010) used digital images to analyze the morphometry of some fossil seeds of *Vitis*, recovered from the Gray Fossil Site in northeastern Tennessee, USA, and datable to latest Miocene-earliest Pliocene. On the basis of 11 measured parameters, they placed the seeds in three different morphotaxa.

Using digital images, Bacchetta et al. (2008) characterized seeds of wild plants typical of the Mediterranean basin, implementing statistical classifiers able to discriminate seeds belonging to different genera and species, and achieving promising results. This system was later improved, adding 20 new morphometric and colorimetric features (Mattana et al. 2008). Recently, Grillo et al. (2010) published the results of the use of statistic classifiers, based on morphometric and colorimetric features of seeds, for ten of the most representative families of the Mediterranean vascular flora, confirming the validity of the method.

Currently, this method is fully accepted and utilized, not only for archaeological studies or taxonomic investigations of wild species (Bacchetta et al. 2011a, b), but also to study cultivated plants, to compare different varieties, contributing to the cataloguing and

conservation in germplasm banks, or allowing the definition of objective parameters for the typifying of particular landraces in the attribution of European trademarks such as protected designation of origin (PDO) and protected geographical indication (PGI) (Grillo et al. 2011; Kiliç et al. 2007; Venora et al. 2007, 2009a, b). All these studies prove that the morphological traits of the seed, such as shape, size and external ornamentations represent very important diagnostic factors in plant taxonomy studies. Moreover, the increasing availability of seeds of wild plants from archaeological sites and above all those stored in seed banks, emphasizes the importance of seed macro and micro morphology studies in plant taxonomy (Grillo et al. 2010).

This paper proposes an accurate identification approach for recognizing archaeological seeds belonging to the genus *Vitis,* based on characterization by image analysis. In particular, the aims of this study are:

1) to assemble a database of morphological parameters and EFDs to characterize the collected seeds belonging to *V. v. vinifera* and *V. v. sylvestris*;

2) to compare the archaeological seeds with the recent seeds of both species based on the established database, to identify the relationships between the archaeological unknown seeds and *V. v. vinifera* and *V. v. sylvestris* materials, using Linear Discriminant Analysis (LDA).

4.3. Materials & Methods

4.3.1. Archaeological seed materials

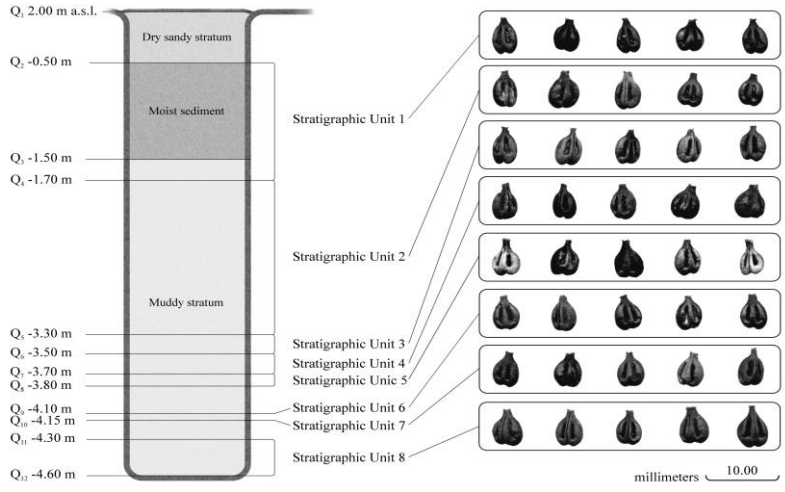

Figure 1. Seed of *Vitis* L dug up from eight different stratigraphic units ranged between -0,5 m and -4,60 m, in the shaft N of the prenuragic and nuragic complex of Sa Osa.

. Seeds from two Sardinian archaeological sites were available, and were selected on the basis of preservation state. 790 selected and waterlogged seeds of *Vitis,* (7 samples of the best 100 seeds, 1 with only 90 seeds) coming from eight different stratigraphic units between -0.5 m and -4.60 m, in the shaft N of the

pre-Nuragic and Nuragic complex of Sa Osa in central western Sardinia (39°55’16.50’’N; 8°32’46.76’’E), belonging to the Middle and Final Bronze Age (Fig. 1) and dated to 1300-1200 B.C. according to Depalmas (2009), and 1600-1200 B.C. according to Sanges (2010). Six carbonized seeds found inside the Phoenician amphora 78A2, from near Isola di Coltellazzo, Sardinia (38°59’02.00’’N; 9°01’17.78’’E), and dated to 600-300 B.C., were selected too (Fig. 2).

The selected seed lots were kept in plastic containers of 50 ml in deionised water. Before image acquisition, they were they placed on tissue paper to absorb excess water, carefully cleared with a brush, and scanned in sub-lots of 10 seeds. After image acquisition, the sample lots were saved in single packets and then grouped into hermetic bottles of 500 ml in deionised water. Each bottle was stored in the dark at 5-10°C.

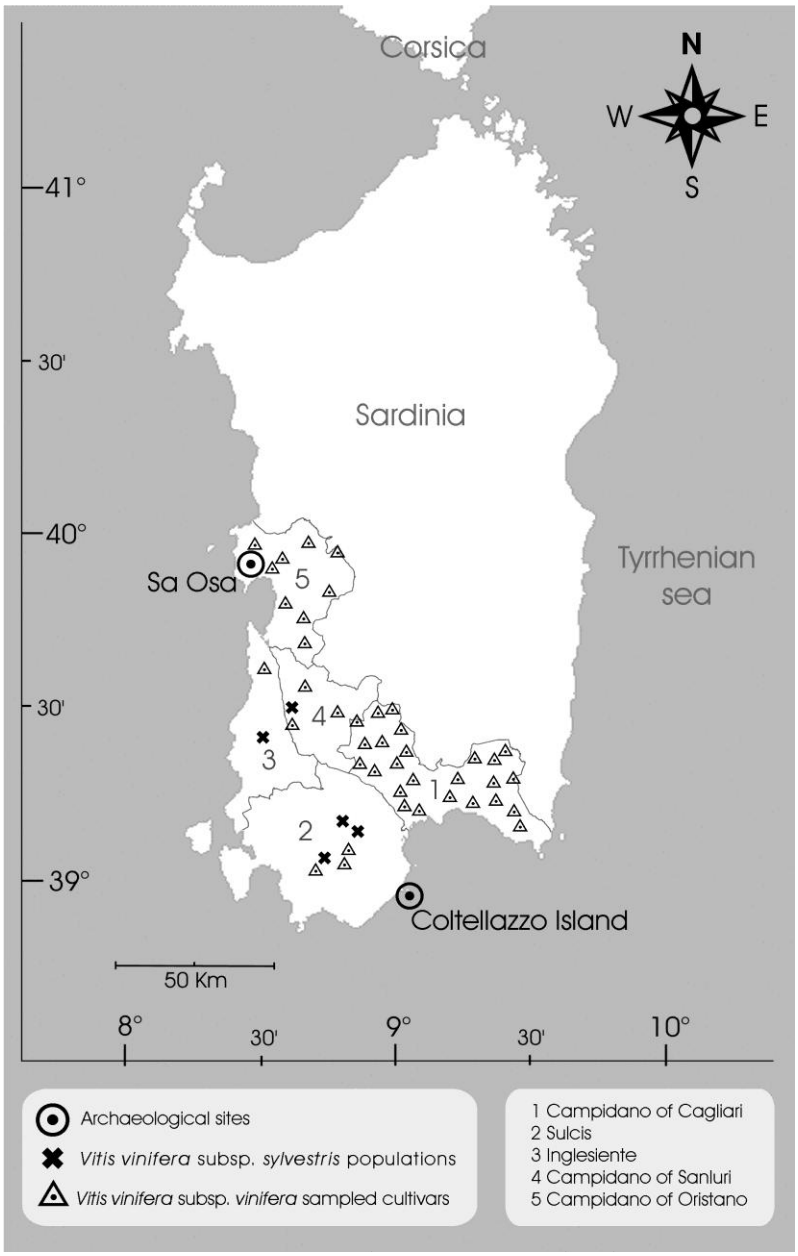

Figure 2. Distribution map of Archaeological sites, *V. v. sylvestris* population and *V. v. vinifera* sampled cultivar in Sardinia.

4.3.2. Modern seed material

A total of over 4000 seeds of the five most representative and best preserved populations of *V. v. sylvestris* were collected throughout southwest Sardinia from along riverbanks or colluvial sites on hilly humid slopes (Table 1 and Fig. 2), and fruits/seeds of 37 Sardinian cultivars, from southern and central western Sardinia, two Italian and two French cultivars of V. vinifera were selected from modern populations developed and maintained in the experimental farms of Ussana by the Agenzia per la Ricerca Scientifica della Regione Sardegna (AGRIS) in total more than 38,000 diaspores (Table 2).

Table 1. Collecting localities, substrata typology, bioclimatic data, altitude range, terrestrial coordinates, collected seeds and number of individuals for each population of *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel) Hegi studied populations.

Localities	Substrata	Termotype and ombrotype	Altitude range m a.s.l.	Coordinates		Seed amount	Number of individuals
				N	E		
Siliqua (CA)	Metamorphytes	Thermomedit. sup. Dry sup.	119-183	39°14'03.1"	8°48'53.8"	361	11
Santadi (CI)	Metamorphytes	Thermomedit. sup. Dry sup.	178-418	39°03'17.8"	8°46'22.6"	728	92
Uta (CA)	Granites	Thermomedit. sup. Dry sup.	30-115	39°11,7'14"	8°56'00.0"	1358	271
Fluminimaggiore (CI)	Metamorphytes	Mesomedit. inf. Subhumid inf.	130-270	39°24'02.2"	8°28'22.8"	1422	61
Gonnosfanadiga (VS)	Metamorphytes	Mesomedit. Inf. Subhumid inf.	337-479	39°28'59.7"	8°34'49.0"	392	22

Table 2. Variety name, grape colour and distribution of *Vitis vinifera* L. subsp. *vinifera* studied cultivars.

Grape variety	Grape colour	Distribution
Alicante	Black	France
Argu mannu	White	Sardinia
Axina de Francia	White	Campidano of Cagliari
Axina de tres bias	Black	Campidano of Cagliari
Bianca di Mara	White	Campidano of Cagliari
Bovali mannu	Black	Campidano of Cagliari
Caddiu	Black	Campidano of Cagliari
Cannonau bianco	White	Campidano of Cagliari
Carenisca	Black	Sulcis
Carignano	Black	Sulcis
Codronisca	White	Campidano of Cagliari
Gabriella	Black	Campidano of Cagliari
Gioia bella	White	Campidano of Cagliari
Girò di Gonnos	Black	Campidano of Sanluri
Gregu biancu	White	Campidano of Cagliari and Oristano
Gregu nieddu	Black	Campidano of Cagliari and Oristano
Grillu	White	Campidano of Cagliari
Licronaxiu	White	Campidano of Oristano
Licronaxiu bianco	White	Campidano of Oristano
Licronaxiu nero	Black	Campidano of Oristano
Malaga	Black	Sardinia
Manzesu	Black	Campidano of Cagliari
Mizu	White	Campidano and Marmilla
Monica nera	Black	Campidano of Cagliari
Moscatello bianco	White	Campidano
Moscatello nero	Black	Campidano of Cagliari
Nasco	White	Campidano of Cagliari
Nieddera	Black	Campidano of Oristano
Nuragus	White	Campidano of Cagliari
Pinot bianco	White	France
Rosa di Mara	Black	Campidano of Cagliari
Salude e passa	Black	Campidano of Cagliari
Sangiovese	Black	Tuscany
Tintillu	Black	Campidano of Cagliari
Tittiacca di Gonnos	White	Campidano of Sanluri
Tittiacca rosa	White	Campidano of Cagliari
Tittiacca verde	White	Campidano of Cagliari
Trebbiano romagnolo	White	Romagna
Vernaccia	White	Campidano
Vernaccia di Solarussa	White	Campidano of Oristano
Vertudi	Black	Sulcis

4.3.3. Seed size and shape analysis

Digital images of the modern seed samples were acquired using an Epson GT 15000 flatbed scanner with a digital resolution of 200 dpi and a scanning area not exceeding 1024×1024 pixels. Image acquisition was performed before drying the seeds at 15°C to 15% of R.H. to avoid spurious variation in dimension, shape and colour. Before image acquisition, the scanner was calibrated for colour matching following the protocol of Shahin and Symons (2003), as suggested by Venora et al. (2009a).

Samples consisting of 100 seeds were captured and used for the digital image analysis. In order to represent the whole variability of each of the modern seed lots, the seed samples

were scanned three times, randomly disposing them each time on the flatbed tray. A total of over 42,000 statistical cases were analysed.

The archaeological seeds were scanned differently and they were put on the scanner in organized columns of 10 by 10 and labelled with univocal numbers, to allow their identification during the analysis. Moreover, due to the irregular shape of these seeds, both the dorsal and ventral faces were scanned, in order to consider the whole morphological variability of each sample and contextually to increment the number of statistical cases.

The digital images of the seeds were processed and analysed using the software package KS-400 V. 3.0 (Carl Zeiss, Vision, Oberkochen, Germany). A macro specifically developed for the characterization of wild seeds (Bacchetta et al. 2008), and later modified to measure a further 20 morpho-colorimetric seed features (Mattana et al. 2008), was adapted to perform all the analysis procedures automatically, reducing the execution time and contextual mistakes in the analysis process (Grillo et al. 2010). Due to the unsuitable colorimetric features of the archaeological seed lots, only 13 features descriptive of seed size and shape were measured (Table 3).

Moreover, because of the few available features descriptive of seed size and shape, the binary images obtained by the segmentation process during the image analysis of the seeds were redefined to 400 dpi to enhance the image definition and apply the EFD method, to increase the number of discriminant parameters (Bacchetta et al. 2009, 2010). This method allows description of the boundary of the seed projection, as an array of complex numbers which correspond to the pixel positions on the seed boundary. So, from the seed apex, 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 along the boundary 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 boundary (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 outlines, are used to standardize size (surface area) and to orientate seeds (Terral et al. 2010). According to Terral et al. (2010), about the use of 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

boundaries, obtaining a further 80 parameters useful to discriminate between the studied seeds of *Vitis*.

Table 3 - List of the 13 morphometric features measured on seeds, excluding the 80 Elliptic Fourier Descriptors.

	Feature	**Description**
$\boldsymbol{A}$	Area	Seed area (mm^2)
$\boldsymbol{P}$	Perimeter	Seed perimeter (mm)
$\boldsymbol{P_{conv}}$	Convex Perimeter	Convex perimeter of the seed (mm)
$\boldsymbol{P_{Crof}}$	Crofton's Perimeter	Perimeter of the seed calculated using the Crofton's formula (mm)
$\boldsymbol{P_{conv}/P_{Crof}}$	Perimeter ratio	Ratio between convex and Crofton's perimeters
$\boldsymbol{D_{max}}$	Max diameter	Maximum diameter of the seed (mm)
$\boldsymbol{D_{min}}$	Min diameter	Minimum diameter of the seed (mm)
$\boldsymbol{D_{min}/D_{max}}$	Feret ratio	Ratio between minimum and maximum diameters
$\boldsymbol{Sf}$	Shape Factor	Seed shape descriptor = (4 x π x area)/perimeter^2 (normalized value)
$\boldsymbol{Rf}$	Roundness Factor	Seed roundness descriptor = (4 x area)/(π x max diameter^2) (normalized value)
$\boldsymbol{Ecd}$	Eq. circular diameter	Diameter of a circle with an area equivalent to that of the seed (mm)
$\boldsymbol{EA_{max}}$	Maximum ellipse axis	Maximum axis of an ellipse with equivalent area (mm)
$\boldsymbol{EA_{min}}$	Minimum ellipse axis	Minimum axis of an ellipse with equivalent area (mm)

4.3.4. Statistical analysis

The obtained data from modern *V. v. sylvestris*, *V. v. vinifera* and the archaeological *Vitis* seeds built up a database. The data were statistically elaborated applying the stepwise Linear Discriminant Analysis (LDA) method by using the SPSS software package release 15 (SPSS Inc. 1989-2006), to compare the modern cultivars with the archaeological seeds considered as unidentified cases. This approach is commonly used to classify or identify unknown groups characterized by quantitative and qualitative variables (Fisher 1936, 1940). On the basis of all measured features, the stepwise method identifies and selects the best of them to use for the seed sample identification, using three statistical variables, Tolerance, F-to-enter and F-to-remove. The Tolerance value indicates the proportion of a variable variance not accounted for by other independent variables in the equation. A variable with very low Tolerance values provides little information to a model. F-to-enter and F-to-remove values

define the power of each variable in the model and they are useful to describe what happens if a variable is inserted and removed, respectively, from the current model (Bacchetta et al. 2010). This method starts with a model that does not include any of the variables. At each step, the variable with the largest F-to-enter value that exceeds the entry criteria chosen ($F \geq 3.84$) is added to the model. The variables left out of the analysis at the last step have F-to-enter values smaller than 3.84, so no more are added. The process was automatically stopped when no remaining variables increased the discrimination ability (Venora et al. 2009b).

Finally, a cross-validation procedure was applied to verify the performance of the identification system, testing individual unknown cases and classifying them on the basis of all others (SPSS release 15, SPSS Inc. 1989-2006).

4.4. Results

A total of 93 morphological quantitative variables describing seed size and shape were measured and then analysed by stepwise LDA, to implement statistical classifiers able to distinguish the studied cases. For each classifier, the stepwise method chooses between 42 and 64 variables among the 93 available to classify the seed groups. Although the perimeter ratio (Pconv /PCrof) was always the first, the second or the third feature selected by the model on the basis of the discriminatory power, showing high values of F-to-remove (data not shown), all the discrimination processes were carried out principally by EFDs. For each implemented classifier, seven or eight EFDs were present among the first ten chosen parameters. Maximum diameter (Dmax), Feret ratio (Dmin/Dmax), Equivalent circular diameter (Ecd) and Convex perimeter (Pconv) provided other powerful features for the discrimination model (data not shown, Table 3).

Using this model, a preliminary comparison among the seeds of *V. v. vinifera*, *V. v. sylvestris* and those from the two archaeological sites considered as an unique unknown group was executed, achieving, for the archaeological seeds, grouping percentages of 58.7% and 41.3% in *V. v. vinifera* group and *V. v. sylvestris* group, respectively (Table 4).

Table 4. Correct classification percentage between Vitis vinifera L. subsp. sylvestris (C.C. Gmel) Hegi and Vitis vinifera L. subsp. vinifera and grouping percentages of archaeological seeds considered as unknown. In parenthesis, the number of analysed.

	Vitis vinifera* Subsp. *vinifera	***Vitis vinifera* subsp. *sylvestris***	**Total**
Vitis vinifera* subsp. *vinifera	80.8 (1,205)	19.2 (286)	100 (1,491)
Vitis vinifera* subsp. *sylvestris	24.5 (9,537)	75.5 (29,392)	100 (38,929)
Archaeological seeds as unknown	**58.7 (889)**	**41.3 (626)**	100 (1,515)
Overall			**80.6 (41,935)**

Afterwards, it was possible to compare the eight seed lots of *Vitis* from the eight stratigraphic units of the pre-Nuragic and Nuragic complex of Sa Osa, to evaluate the relationship among them (Table 5, Fig. 1). As shown in Table 5, only 29.3% of the seeds belonging to different stratigraphic levels were correctly identified, and none of the eight stratigraphic units is predominant to the others, for this reason they were considered as a unique sample lot in the following elaborations.

Table 5. Correct classification percentage of the archaeological seed lots of *Vitis* from the eight stratigraphic units of the prenuragic and nuragic complex of Sa Osa. In parenthesis, the number of analysed seeds.

Stratigraphic unit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Total**
1	**36.0 (36)**	28.0 (28)	7.0 (7)	8.0 (8)	4.0 (4)	3.0 (3)	6.0 (6)	8.0 (8)	100.0 (100)
2	21.0 (21)	**43.0 (43)**	4.0 (4)	9.0 (9)	5.0 (5)	4.0 (4)	7.0 (7)	7.0 (7)	100.0 (100)
3	8.0 (8)	4.0 (4)	**37.0 (37)**	8.0 (8)	22.0 (22)	5.0 (5)	8.0 (8)	8.0 (8)	100.0 (100)
4	11.0 (10)	16.0 (14)	12.0 (11)	**29.0 (27)**	6.0 (5)	9.0 (8)	8.0 (7)	9.0 (8)	100.0 (90)
5	7.0 (7)	2.0 (2)	25.0 (25)	6.0 (6)	**31.0 (31)**	8.0 (8)	18.0 (18)	3.0 (3)	100.0 (100)
6	6.0 (6)	17.0 (17)	11.0 (11)	6.0 (6)	20.0 (20)	**13.0 (13)**	20.0 (20)	7.0 (7)	100.0 (100)
7	5.0 (5)	12.0 (12)	19.0 (19)	9.0 (9)	11.0 (11)	14.0 (14)	**25.0 (25)**	5.0 (5)	100.0 (100)
8	18.0 (18)	9.0 (9)	19.0 (19)	11.0 (11)	8.0 (8)	7.0 (7)	8.0 (8)	**20.0 (20)**	100.0 (100)
Overall									**29.3 (790)**

Then, the seeds of the two archaeological sites were compared with the populations of *V. v. sylvestris*, achieving an overall cross-validated performance of correct classification of 97.6 % (Table 6). The seeds found in the shaft N of the Sa Osa complex were correctly classified in 97.7% of the cases, while 66.7% of the seeds from the Phoenician amphora 78A2 near Isola di Coltellazzo were correctly attributed. Also, V. sylvestris was recognized with a

high percentage the correct identification and only a few seeds were mistaken for archaeological seeds and vice versa.

Table 6. Correct classification percentage between *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel) Hegi and archaeological seed lots. In parenthesis, the number of analysed seeds.

	Vitis vinifera* subsp. *sylvestris	**Seed lots from Sa Osa**	**Seed lots from Coltellazzo Island**	**Total**
Vitis vinifera* subsp. *sylvestris	**97.7 (1,456)**	2.2 (33)	0.1 (2)	100 (1,491)
Seed lots from Sa Osa	2.3 (35)	**97.7 (1,474)**	-	100 (1,509)
Seed lots from Coltellazzo Island	16.7 (1)	16.7 (1)	**66.7 (4)**	100 (6)
Overall				**97.6 (3,006)**

From a more detailed comparison among the seeds of the two archaeological sites and the five populations of *V. v. sylvestris*, an overall percentage of correct identification of 82.2% was achieved (Table 7). The seeds of *V. v. sylvestris* populations were distributed into the *V. v. sylvestris* group, in which correct classification performances are included between 48.0% and 64.5%, except the population of Siliqua which showed a percentage of correct identification of 93.0%; while 98.5% and 50.0% of the archaeological seed lots from Sa Osa and Isola di Coltellazzo respectively, were correctly identified.

Table 7. Correct classification percentage between *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel) Hegi populations and archaeological seed lots. In parenthesis, the number of analysed seeds.

	Siliqua	**Santadi**	**Uta**	**Fluminimaggiore**	**Sibiri**	**Seed lots from Coltellazzo Island**	**Seed lots from Sa Osa**	**Totali**
Siliqua	**93.0 (279)**	-	6.7 (20)	0.3 (1)	-	-	-	100 (300)
Santadi	0.3 (1)	**48.0 (143)**	15.1 (45)	13.8 (41)	19.8 (59)	-	3.0 (9)	100 (298)
Uta	2.4 (7)	13.1 (39)	**61.3 (182)**	10.8 (32)	7.4 (22)	-	5.1 (15)	100 (297)
Fluminimaggiore	2.0 (6)	15.5 (46)	13.1 (39)	**62.3 (185)**	4.0 (12)	-	3.0 (9)	100 (297)
Sibiri	-	13.7 (41)	14.0 (42)	7.4 (22)	**64.5 (193)**	-	0.3 (1)	100 (299)
Seed lots from Coltellazzo Island	-	-	-	33.3 (2)	-	**50.0 (3)**	16.7 (1)	100 (6)
Seed lots from Sa Osa	0.6 (9)	0.3 (5)	0.4 (6)	0.1 (2)	-	-	**98.5 (1,487)**	100 (1,509)
Overall								**82.2% (3,006)**

Comparing the two archaeological seed assemblages with the cultivars of V. vinifera, an overall cross-validated performance of 98.2% was achieved (Table 8). The archaeological seeds from the Sa Osa complex reached a percentage of correct identification of 79.5%, in

which misidentified seeds were exclusively mistaken for *V. v. vinifera*, while the seeds from the Isola di Coltellazzo were correctly classified in 50.0% of the cases, and also in this case the misclassified seeds were only mistaken for V. vinifera. The correct identification percentage of *V. v. vinifera* seeds was very high (99.0%), with modest numbers of seeds wrongly classified into both archaeological groups.

Table 8. Correct classification percentage between *V. vinifera* L. subsp. *vinifera* and archaeological seed lots. In parenthesis, the number of analysed seeds.

	V. vinifera* subsp. *vinifera	**Seed lots from Sa Osa**	**Seed lots from Coltellazzo Island**	**Total**
V. vinifera* subsp. *vinifera	**99.0 (38,525)**	0.8 (294)	0.3 (110)	100 (38,929)
Seed lots from Sa Osa	20.5 (309)	**79.5 (1,200)**	-	100 (1,509)
Seed lots from Coltellazzo Island	50.0 (3)	-	**50.0 (3)**	100 (6)
Overall				**98.2 (40,444)**

In order to identify a narrower group of *V. v. vinifera* to which the archaeological seed lots could be a closer match, a comparison among the seeds of the two archaeological sites and the *V. v. vinifera* cultivars clustered for the colour of its grapes was done (Table 9). This approach showed a rather low overall performance (58.8%), mainly due to the mistakes between the two *V. v. vinifera* groups. It is important to highlight the distribution of the misidentified seeds of the two archaeological seed lots. The wrongly classified seeds of the archaeological group of Sa Osa were identified as white grape *V. v. vinifera* in 12.7% of the cases, and as black grape *V. v. vinifera* in 4.0% of the cases, while the misidentified seeds of the archaeological seed lot from Isola di Coltellazzo, although consisting of only three seeds, was classified as white grape *V. v. vinifera* in 33.3% of the cases and as black grape V. vinifera in 16.7% of the cases.

Table 9. Correct classification percentage between *Vitis vinifera* L. subsp. *vinifera* and archaeological seed lots distinguished for grape colour. In parenthesis, the number of analysed seeds.

	White grape *V. vinifera* subsp. *vinifera*	**Black grape *V. vinifera* subsp. *vinifera***	**Seed lots from Sa Osa**	**Seed lots from Coltellazzo Island**	**Total**
White grape *V. vinifera* subsp. *Vinifera*	**58.8 (11,737)**	39.8 (7,952)	1.2 (232)	0.2 (49)	100 (19,970)
Black grape *V. vinifera* subsp. *vinifera*	42.0 (7,964)	**56.9 (10,789)**	0.8 (144)	0.3 (62)	100 (18,959)
Seed lots from Sa Osa	12.7 (192)	4.2 (63)	**83.1 (1,254)**	-	100 (1,509)
Seed lots from Coltellazzo Island	33.3 (2)	16.7 (1)	-	**50.0 (3)**	100 (6)
Overall					**58.8 (40,444)**

Finally, comparing the two archaeological sites with the seeds of all the cultivar sample lots of *V. v. vinifera* individually considered, an overall cross-validated performance of 41.0% was achieved (Table 10). The seeds from shaft N of the Sa Osa complex reached a percentage of correct identification of 85.2%. Errors were evenly distributed in more than half of the considered cultivars. Similarly, 50.0% (3 specimens) of the misclassified seeds from the Phoenician amphora were wrongly attributed to three of the *V. v. vinifera* cultivars (Grillu, Licronaxiu and Girò di Gonnos). None of the seeds from the two archaeological sites were mistaken for each other. Regarding the identified *V. v. vinifera* cultivars, low performances were achieved, ranged between 10.9% (Licronaxiu bianco) and 80.6% (Vernaccia), but only ten out of 41 seed lots showed a percentage of correct identification higher than 50.0%.

Table 10. Correct classification percentage between *Vitis vinifera* L. subsp. *vinifera* cultivars and archaeological seed lots. In parenthesis, the number of analysed seeds.

	Cultivars	Archaeological seed lots		
	Vitis vinifera subsp. *vinifera*	Seed lots from Coltellazzo Island	Seed lots from Sa Osa	Total
Seed lots from Coltellazzo Island	50.0 (3)	50.0 (3)	-	100 (6)
Seed lots from Sa Osa	14.8 (223)	-	85.2 (1,286)	100 (1,509)
Alicante	26.8 (160)	-	0.1 (1)	100 (598)
Argu mannu	22.7 (270)	-	0.3 (4)	100 (1,192)
Axina de Francia	44.0 (528)	-	0.2 (3)	100 (1,199)
Axina de tres bias	50.3 (598)	-	-	100 (1,189)
Bianca di Mara	32.2 (431)	-	0.1 (2)	100 (1,337)
Bovali mannu	34.4 (407)	-	0.5 (7)	100 (1,184)
Caddiu	54.0 (644)	-	-	100 (1,192)
Cannonau bianco	51.3 (306)	-	2.1 (32)	100 (596)
Carenisca	35.5 (369)	-	0.3 (5)	100 (1,038)
Carignano	40.0 (358)	-	0.1 (1)	100 (895)
Codronisca	50.9 (531)	-	2.3 (34)	100 (1,044)
Gabriella	39.0 (234)	-	0.1 (1)	100 (600)
Gioia bella	22.7 (135)	-	0.1 (2)	100 (595)
Girò di Gonnos	19.6 (116)	16.7 (1)	0.1 (1)	100 (593)
Gregu biancu	15.7 (163)	-	-	100 (1,041)
Gregu nieddu	44.4 (532)	-	0.8 (12)	100 (1,199)
Grillu	61.0 (735)	16.7 (1)	-	100 (1,204)
Licronaxiu	40.5 (423)	16.7 (1)	0.3 (4)	100 (1,045)
Licronaxiu bianco	10.9 (65)	-	0.5 (7)	100 (595)
Licronaxiu nero	24.2 (145)	-	0.3 (5)	100 (600)
Malaga	21.1 (127)	-	0.1 (1)	100 (601)
Manzesu	40.7 (854)	-	0.2 (3)	100 (2,098)
Mizu	33.9 (712)	-	1.1 (16)	100 (2,098)
Monica nera	56.7 (340)		-	100 (600)
Moscatello bianco	52.3 (627)	-	0.5 (7)	100 (1,199)
Moscatello nero	31.1 (325)	-	1.4 (21)	100 (1,044)
Nasco	25.7 (192)		0.1 (2)	100 (746)
Nieddera	40.0 (479)	-	-	100 (1,197)
Nuragus	21.3 (189)	-	-	100 (888)
Pinot bianco	30.9 (185)	-	0.3 (5)	100 (599)
Rosa di Mara	35.5 (319)	-	-	100 (899)
Salude e passa	46.2 (274)	-	0.9 (14)	100 (593)
Sangiovese	25.2 (150)	-	-	100 (596)
Tintillu	38.0 (397)	-	0.3 (5)	100 (1,045)
Tittiacca di Gonnos	59.4 (336)	-	-	100 (566)
Tittiacca rosa	48.2 (286)	-	0.1 (1)	100 (593)
Tittiacca verde	60.0 (356)	-	-	100 (593)
Trebbiano romagnolo	62.2 (371)	-	1.0 (15)	100 (596)
Vernaccia	80.6 (961)	-	-	100 (1,193)
Vernaccia di Solarussa	24.1 (253)	-	0.1(2)	100 (1,049)

4.5. Discussion and conclusion

Many times morpho-colorimetric seed characterization by image analysis has proved to be a repeatable, reliable and non-destructive method able to accurately identify seeds both of cultivated (Grillo et al. 2011; Venora et al. 2007; Zapotoczny et al. 2008) and of wild plant species (Bacchetta et al. 2008, 2011a, b; Grillo et al. 2010; Mattana et al. 2008).

Because of the non-representative colour features of the archaeological seeds, in this work, only the morphological characterization of seeds was applied to comparison of the archaeological seed lots with cultivars of *V. v. vinifera* and wild populations of *V. v. vinifera*. Concerning this, the EFDs proved to be very helpful to discriminate between the studied cases, as proved by many authors studying seeds (Iwata et al. 2010; Ohsawa et al. 1998) as well as other plant anatomical traits (Kawabata et al. 2009; Hâruţa 2011; Yoshioka et al. 2007).

From the comparison between the seeds of *V. v. vinifera*, *V. v. sylvestris* and those from the two archaeological sites, a slight but not explicit similarity of the archaeological seeds with the *V. v. vinifera* group was highlighted. Presumably, such a result could be due to the great morphological variability within each group, and on the basis of this hypothesis, separate comparisons for *V. v. vinifera* and *V. v. sylvestris* were subsequently implemented.

Considering the archaeological seeds of *Vitis* from the eight stratigraphic units of the Sa Osa complex, there are almost no differences evident. Therefore and according to the studies conducted by the Soprintendenza per I Beni Archeologici per le provincie di Cagliari e Oristano on the different stratigraphic units (Usai 2011; Sanna 2011), in this study the seeds of the eight stratigraphic levels were considered as a unique seed lot when compared with the *V. v. vinifera* and *V. v. sylvestris* seed lots.

From the comparison of both archaeological seed lots with the wild populations of *V. v. sylvestris*, a clear morphological differentiation between them and the wild populations of *V. v. sylvestris* was revealed. Similar results are obtained when the seed lots of *V. v. sylvestris* were considered as individual populations. A more accurate evaluation of the results shows that the seeds of *V. v. sylvestris* populations were widely distributed into the *V. v. sylvestris* group. Except the population of Siliqua that showed a good performance of correct identification (93.0%), the misidentification distribution among the other wild populations of *V. v. sylvestris* suggests that the numerical size of each wild population may play an important role. Actually, the population of Siliqua, made up of only 11 individuals, five females and six males, is very small compared with all the others. Consequently, the low intra-population variability of Siliqua could explain the modest morphological diversification of the seeds of

this population. In addition, the geographical isolation of the Siliqua population, located far away both from urban areas and from farmlands, in an extreme and wild region, indicates the low probability that intraspecific hybridization phenomena occurred. According to Zecca et al. (2010) wild and domesticated Sardinian grapevines have essentially remained reproductively isolated.

Following the same criteria, the two archaeological seed lots were compared with the seeds of the cultivars of *V. v. vinifera*, showing that a certain relationship probably exists between them. Both archaeological seed lots were widely misidentified as *V. v. vinifera*, and the seeds of the two archaeological groups were never mistaken each other. The comparison between the archaeological seeds and the studied cultivars, split into two groups on the basis of the grape colour, gives hints that the archaeological seed lots seem to be closer to the white grape cultivars than to the black ones.

Finally, comparing the two archaeological seed lots with all the studied cultivars individually, it is possible to notice that four of the first five bigger mistakes with the seed lots from the Sa Osa complex are related to white grape cultivars (Cannonau bianco, Codronisca, Mizu and Trebbiano Romagnolo), achieving for them a percentage of misidentification of 6.5%. A similar consideration can be done on the basis of the misclassified seeds from Isola di Coltellazzo, for which two of the three misidentifications are related to white grape cultivars (Grillu and Licronaxiu).

The results obtained by establishing a general database of morphological features of the genus *Vitis* and the implementation of a seed classifier for *V. v. vinifera* and *V. v. sylvestris* species groups, prove once again how image analysis techniques can be considered as a useful tool not only in taxonomic investigations, but also in archaeobotanical studies. Moreover, the adoption of the EFDs as discriminant parameters proves to be very important, especially when colour parameters are not applicable, although these were important when dealing with modern material. Regarding the results, the domestication and selection work by humans, the high degree of intraspecific hybridization of *V. v. vinifera*, the low probability of interspecific hybridization phenomena, the annual biological cycle and above all the very long period that separates the ancient from the modern seed lots considered in this study, are very important factors to consider. Surely all these elements influenced and contributed to determine the evolution of modern cultivars, contextually causing the loss of ones that currently should be very close to the archaeological seeds. This assumption makes the achieved results certainly considerable. Moreover, the obtained high level of dissimilarity is plausible also considering

the impossibility of using the colorimetric features, which many times proved to be very powerful parameters for the identification process.

The application of image analysis allowed identification of the relationship between the seed lots from the archaeological sites of Sa Osa and Isola di Coltellazzo, and the modern cultivars historically grown close to the archaeological sites, and the wild populations of *V. v. sylvestris* collected near the two sites. In particular, the analysis of the archaeological seeds of *Vitis* from the eight stratigraphic units of the shaft N of pre-Nuragic and Nuragic complex of Sa Osa could show that the seeds are very similar, confirming that the different stratigraphic units belong to the same period (Middle and Final Bronze Age).

Taking into consideration the difference between the two kinds of archaeological seeds, it becomes likely that the archaeological seeds from the Phoenician amphora came from different cultivation areas, far away from Sardinia. If so, the similarity with white grape cultivars should be merely coincidental. A larger database including more cultivars and sample lots may allow a more accurate identification in the future.

Anyway, the greater similarity of the archaeological seeds to *V. v. vinifera* cultivars than to *V. v. sylvestris* populations, and especially to white grapes rather than black grape cultivars, could prove that in the Campidano in southern Sardinia, white grapes were probably already used at 1600-1200 B.C., explaining that it may be not a chance that white grapes are still traditionally cropped today in the Campidano area to produce famed wines, as well as in the Vernaccia.

According to the analysis conducted by Lovicu et al. (2011), the seeds at Sa Osa could be identified as V. vinifera, allowing us to affirm that, between the Middle and Final Bronze Age, very similar varieties of *V. v. vinifera* were used to produce wine or to preserve as foodstuffs, although the production, attested in the Nuragic period thanks to the find of a Nuragic wine press in Monastir in southern Sardinia) close to Monte Zara, is dated between 900 and 800 B.C., the recent Iron Age (Ugas 1999). Considering that all the previous Sardinian remains of *Vitis* seeds cited in literature were dated to the Final Bronze Age, and looking at the dating of all the other finds in the Italian peninsula and in the Mediterranean area (Aranguren et al. 2007; Bellini et al. 2008; Buxó and Capdevila 1997; Costantini 1981; Delpino 2007; Forni 2007; Martinoli 2004; McGovern 2003a; McGovern 2003b; McGovern 2004; Vaquer 1986), according to Sanges (2010) the seeds from the pre-Nuragic and Nuragic complex of Sa Osa are the oldest remains of *Vitis* found in Sardinia, the oldest find of domesticated *Vitis* in Italy and among the most ancient remains of domesticated Vitis found in the Mediterranean area.

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5. Computer vision as a complementary to molecular analysis: grapevines cultivars case study

5.1. Abstract

Despite the different breeding events as well as the domestication phenomena contributed to enrich the grape varietal heritage in Sardinia, a lot of local varieties simply are the product of linguistic distorting due to the wide heterogeneity historic-cultural of the island. This phenomena generated a great assortment of grape names, that, together with the huge real number of cultivars, is the cause of the incredible current grapevine Sardinian panorama.

The goal of this paper is to compare the published molecular data of 40 Sardinian autochthonous cultivars with the results achieved by the germplasm phenotypical characterization, on the basis of morpho-colorimetric features and Elliptic Fourier Descriptors (EFDs), measured by image analysis. Statistical classifiers were implemented to discriminate dissimilar seeds and carry out hypothetical synonymy groups to compare with those proposed on the basis of Simple Sequence Repeat (SSR) markers. This work represents the first trial to validate a morpho-colorimetric characterization method by direct comparison with molecular data, proving that the 113 measured features of the germplasm resulted adequate to achieve a clear discrimination among the synonymy groups.

5.2. Introduction

The domestic grape represents one of the oldest crop in the world (Zecca et al. 2010), with remarkable importance both from a cultural and economic point of view due to its transformation in wine (Manen et al. 2003, This et al. 2006, Vivier & Pretorius 2002). According to the data published by FAO (2007), currently 71% of world grape production is used for wine making, 27% as fresh fruit and 2% as dried fruit.

Such product was once widespread only among the Mediterranean populations, while today it is a popular asset commonly traded in the global marker (Manen et al. 2003, This et al. 2006).

Nevertheless about 10.000 cultivars have been recorded among the *Vitis vinifera* L. species, substantial information has been gained by molecular studies regarding more than 5.000 varieties, highlighting wrong nomenclatural attributions due to synonymy and/or homonymy cases for a lot of cultivars (This et al. 2006).

Sardinia, with a dimension of 24.084 Km^2, is the second largest island in the Mediterranean Sea and represents an interesting laboratory, from biological, historical and cultural perspectives, where to address studies on the characterization of the huge wine-producing heritance, currently attested on 151 cultivars (Lovicu et al. 2010).

The typical insular condition of Sardinia and the large population of wild grapevine coenosis distributed in the whole island (Grassi et al. 2008), allowed the local populations to develop independent domestication processes. Similar phenomena has also been recorded in other regions of the Mediterrenean basin, providing considerable support on the polycentric origin theory of the grape (Arroyo García et al. 2002, 2006, Imazio et al. 2006).

An unequivocal evidence that Sardinia is a secondary domestication area has been found in the countryside around Nuoro. By molecular studies, Grassi et al. (2003),

emphasized how the genetic heritance of 2 autochthonous cultivars (Bovale Muristellu and Bovale Murru) is close to the wild grapevine found in the same locality.

The great amount of cultivars, analysed as local varieties, is not only the product of the direct domestication process of wild grapevine, but it is also the result of crosses among local varieties and plant of spontaneous flora, as well as of the introduction of farm techniques and the importation of cultivars by different foreigner populations landed on the island (De Mattia et al. 2007).

Grapevine seeds are highly polymorphic and have a fundamental role in the taxonomic study within the genus *Vitis* L. (Rivera et al. 2007), in the distribution and domestication processes of the wild grapevine, in many archaeological discoveries (This et al. 2006) and in the study about the identification and grouping of diasporas of *Vitis*.

In recent years, many studies have been directed towards the identification and grouping of diasporas of *Vitis* on the basis of biometric features or morphometric analysis of the biometric parameters. Using an electronic calibre, Rivera et al. (2007) measured 11 morphometric variables on 142 different types of grape: 5 taxa of *Vitis*, 92 cultivars of *V. v. vinifera*, 12 feral/wild populations and 4 hybrids rootstock cultivars. The obtained data were elaborated using a clusters analysis, placing feral/wild populations and related cultivars in their respective clusters, but missing a cluster of wild European grapevine. Applying the Elliptic Fourier Descriptors (EFDs) method, Terral et al. (2010) compared well-preserved archaeological seeds, found in the southern France and dated back to the I B.C., with same European modern cultivars and wild individuals. Also Gong et al. (2010) used digital images to analyze the morphometry of same fossil of *Vitis* seeds, dug up from the Gray Fossil Site (N-E Tennessee, USA) and dated to latest Miocene-earliest Pliocene, placing them in three different morphotaxa on the basis of 11 measured characters. The seeds of 5 *V. v. sylvestris* populations and the 41 autochthonous cultivars of *V. v. vinifera* were compared with the archaeological seeds found inside a shaft of the prenuragic and nuragic complex of Sa Osa

(Sanna 2011, Usai 2011), dated between the final Bronze Age (XVI-XII B.C.) and/or middle Bronze Age (XIII-XII B.C.) (Depalmas 2009, Sanges 2010) and identified as cultivated species (Lovicu et al. 2011). Many times the domestication processes of *Vitis* L. has been associated to the winery processes (McGovern 2003) attested in Sardinia with the finding of a press in Monastir (S-Sardinia), close to Monte Zara, dated between IX and VIII B.C. to the recent Iron Age (Ugas 1999); while the Sa Osa findings might anticipate the date of Sardinian domestication process at final and middle Bronze Age. Orrù et al. (submitted) through the Linear Discriminant Analysis (LDA) of the biometric parameters between the tree groups, proved the highest similarity of the archaeological seeds to the species of the *V. v. vinifera*, and in particular to the white berry cultivars rather than with the black berry cultivars. According to the authors, these findings suggest that the *V. v. vinifera* was probably already used to produce wine and/or to preserve foodstuffs as grape, also supporting the traditional production of white grapes in particular Sardinian areas.

Based on a previous work carried out by De Mattia et al. (2007), which aim was to genotype various Sardinian autochthonous cultivars using 13 Simple Sequence Repeat (SSR) markers, the goal of this paper is to propose the characterization of the same varieties on the basis of morpho-colorimetric features and EFDs of the seed lots, measured by image analysis. In particular, the aims of this study are:

1) to carry out a database of morphological parameters and EFDs to characterize some of *V. v. vinifera* varieties studied by De Mattia et al. (2007);

2) to implement, on the basis of the developed database, a statistical classifier able to compare the analysed varieties;

3) to carry out hypothetical synonymy groups;

4) to compare the achieved groupings with those proposed by De Mattia et al. (2007) using SSR markers.

This work represents the first tentative to validate a morphometric characterization method by direct comparison with molecular data.

5.3. Materials and Methods

5.3.1. Seed material

The seeds of 18 black berry and 22 white berry Sardinian cultivars, among those studied by De Mattia et al. (2007), were collected at the time of natural repining throughout the whole Sardinian region (Table 1 and Fig. 1), for a total of 40 seed accessions of grapevine.

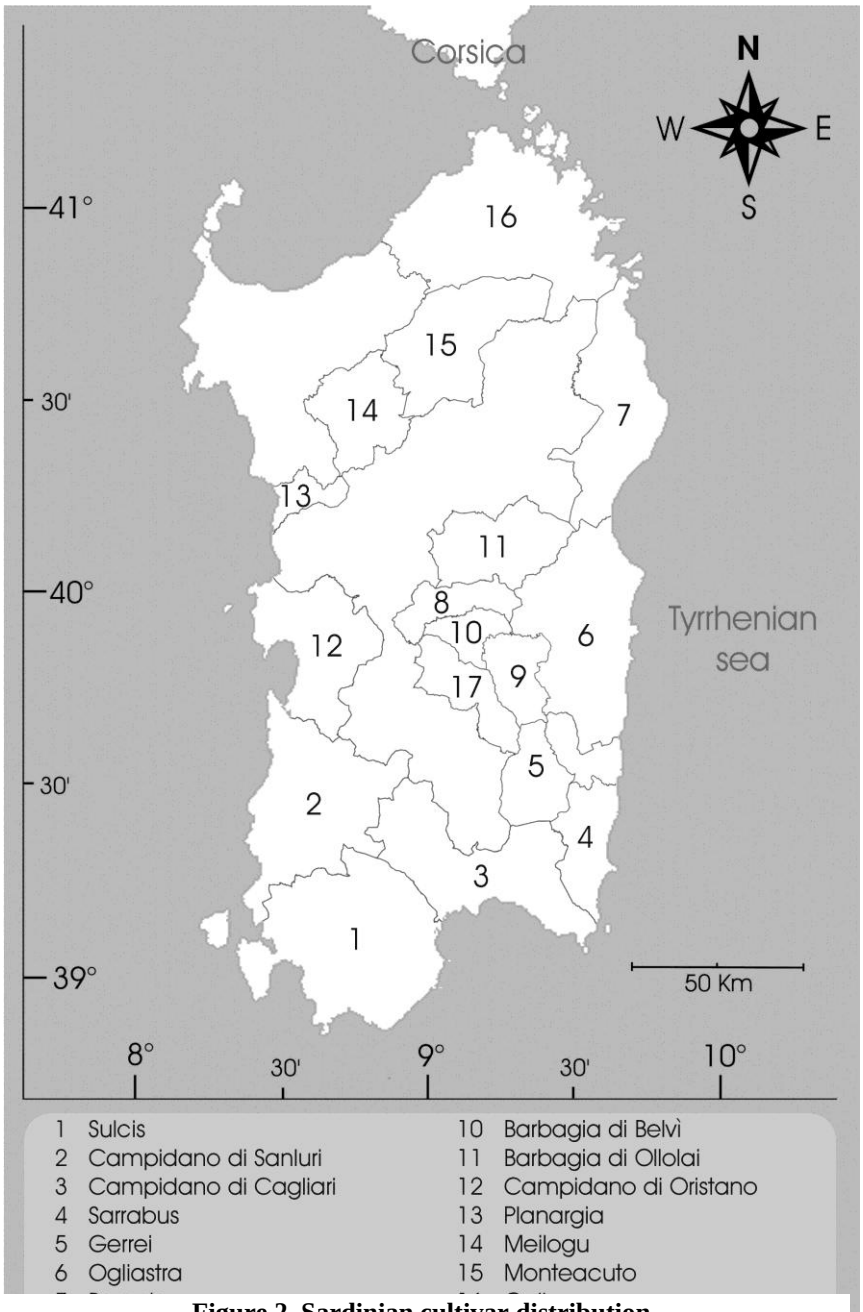

Figure 2. Sardinian cultivar distribution

The necessity to study and characterize vegetal material collected in different localities and diverse ecological conditions, induced the use of different sampling methods, following ethic-scientific criteria (Bacchetta et al. 2006, 2008, Guarino et al. 1995).

Table 1 - Name code, grape variety name, colour (B = black; W = white) and distribution of *V. v. vinifera* L. studied cultivars.

Name code	Grape variety	Grape colour	Distribution
ArB	Aregu Biancu	W	Barbagia di Seùlo
ArG	Aregu Giallo	W	Barbagia di Seùlo
ArM	Argu Mannu	W	Sardinia
Cn	Cannonau	B	Barbagia di Seùlo, Ollolai e Belvì
CnO	Cannonau Bianco di Oliena	W	Barbagia di Ollolai
CnT	Cannonau Bianco di Trieri	W	Ogliastra
Cl	Culupuntu	W	Ogliastra
FlG	Falso Gregu	B	Campidano di Cagliari
GB	Gregu Bianco	W	Campidano di Cagliari e Oristano
GNC	Gregu Nieddu del Campidano	B	Campidano di Cagliari e Oristano
GNS	Gregu Nieddu di Serramanna	B	Campidano di Cagliari
Mz	Manzesu	B	Campidano di Cagliari
ME	Monica di Escalaplano	B	Sarrabus e Gerrei
MSl	Monica di Seulo	B	Barbagia di Seùlo
MSr	Monica di Sorgono	B	Mandrolisai
MLd	Moscato di Lodine	W	Barbagia di Ollolai
MPt	Moscato di Pattada	W	Monteacuto
MT1	Moscato di Tempio 1	W	Gallura
MT2	Moscato di Tempio 2	W	Gallura
MSP	Moscato Su Pinu	W	Barbagia di Ollolai
NrT	Nera Tomentosa	B	Planargia
NrB	Nera di Bosa	B	Planargia
NPd	Nieddu Mannu di Padria	B	Meilogu
NPt	Nieddu Mannu di Pattada	B	Monteacuto
NPS	Nieddu Pedra Serra	B	Campidano di Sanluri
NPl	Nieddu Polchino	B	Monteacuto
Nr	Nuragus	W	Campidano di Cagliari
NrA	Nuragus Arrubiu	W	Campidano di Cagliari
Nrd	Nuragus Muscadeddu	W	Sarrabus e Gerrei
Nrt	Nuragus Moscatello	W	Sarcidano
NrR	Nuragus Rosso Rompizzolla	W	Campidano di Cagliari
PsC	Pascale di Caglairi	B	Campidano di Cagliari
PsO	Pascale di Oliena	B	Barbagia di Ollolai
PrN	Primidivu Nieddu	B	Meilogu
Sn	Sinnidanu	W	Baronie
Vr	Vernaccia	W	Campidano
VrE	Vernaccia di Escalaplano	W	Sarrabus e Gerrei
VrR	Vernaccia di S. Rosalia	W	Ogliastra
VrS	Vernaccia di Solarussa	W	Campidano di Oristano
Vrt	Vertudi	B	Sulcis

5.3.2. Seed analysis

Digital images of seed samples were acquired using a flatbed scanner (Epson GT-15000) with a digital resolution of 400 dpi and a scanning area not exceeding 1024×1024 pixel. Image acquisition was performed before drying the seeds at 15°C to 15% of R.H. to

avoid spurious variation in dimension, shape and colour. Moreover, before image acquisition, the scanner was calibrated for colour matching following the protocol of Shahin and Symons (2003) as suggested by Venora et al. (2009a).

Samples, consisting of 100 seeds, were captured and used for the digital image analysis. In order to represent the whole variability of each accession, the seed samples were acquired three times, randomly disposing them on the flatbed tray. A total of over 32,000 statistical cases were analysed.

Digital images of seeds were processed and analysed using the software package KS-400 V. 3.0 (Carl Zeiss, Vision, Oberkochen, Germany). A macro specifically developed for the characterization of wild seeds (Bacchetta et al. 2008), and later modified to measure further twenty morpho-colorimetric seed features (Mattana et al. 2008), was adapted to perform automatically all the analysis procedures, reducing the execution time and contextually mistakes in the analysis process (Grillo et al. 2010).

Moreover, the binary images obtained by the segmentation process during the image processing of the seeds, were used to apply the EFDs method, to increase the number of discriminant parameters (Bacchetta et al. 2009, 2010) (Tab. 2). As described by Orrù et al. (submitted), this method allows to describe the boundary of the seed projection, as an array of complex numbers which correspond to the pixels position of the seed boundary. According to Terral et al. (2010) findings, about the use of number of harmonics for an optimal description of seed outlines, in order to minimize the measurement errors and optimize the efficiency of shape reconstruction, 20 harmonics were used to define the seed boundaries, obtaining further 80 parameters useful to discriminate among the studied cultivars of *Vitis*.

Table 2 - List of morpho-colorimetric features measured on seeds, excluding the 80 Elliptic Fourier Descriptors (EFDs).

	Feature	**Description**
A	Area	Seed area (mm^2)

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

5.3.3. Statistical analysis

The reached data were used to assemble a database of morpho-colorimetric and EFDs data. Using the SPSS software package release 15 (SPSS Inc. 1989-2006), data were statistically elaborated applying the stepwise LDA method, in order to compare the investigated cultivars. This approach is commonly used to classify/identify unknown groups characterized by quantitative and qualitative variables (Fisher 1936, 1940), finding the combination of predictor variables with the aim of minimizing the within-class distance and maximizing the between-class distance simultaneously, thus achieving maximum class discrimination (Hastie et al. 2001, Holden et al. 2011). On the basis of all measured features, the stepwise method identifies and selects the most statistically significant among them to use for the seed sample identification. This method starts with a model that does not include any of the variables, adding step by step one more, until no remaining variables are able to increase the discrimination ability, stopping the process (Grillo et al. 2011, Venora et al. 2009b).

Finally, a cross-validation procedure was applied to verify the performance of the identification system, testing individual unknown cases and classifying them on the basis of all others (SPSS 1999).

Due to the large number of data, the discriminatory steps were executed distinguishing between black and white berry cultivars, except a preliminary statistical comparisons on the basis of the synonymy groups proposed by De Mattia et al. (2007).

5.4. Results and Discussion

Using the same approach that many times have been applied to solve cases of homonymy and synonymy on the basis of molecular markers, morpho-colorimetric features were used to identify hypothetic grapevine cultivar synonymy groups. A total of 113 morpho-colorimetric features was used to characterize the germplasm of the 40 studied Sardinian grapevine cultivars (Tab. 2).

In order to compare the results of genetic analysis achieved by De Mattia et al. (2007) on Sardinian grapevine cultivars with the relative seed phenotypic expression, a preliminary morpho-colorimetric comparison among 40 of the 61 cultivars tested by De Mattia et al. (2007) was executed on the basis of the 13 synonymy groups proposed by De Mattia et al. (2007). Table 3 shows the results of this preliminary comparison. Although the overall discrimination performance only reached 59.7%, because of the non-inclusion in any group of the 15 cultivars proposed by De Mattia et al. (2007), the percentages of correct identification of each group show a certain correspondence to the phenotypic seed characters (Tab. 3), simultaneously suggesting some potential modifications to the proposed groupings.

From two separate comparisons among black and white berry cultivars (Tabs 4 and 5), only considering the varietal name as grouping variable and excluding the synonymy group notations of De Mattia et al. (2007), evaluating the percentages of correct identification and above all the mistakes made by the classification system, it was possible to identify new hypothetical synonymy groups.

Tab. 3 - Preliminary comparisons among the analysed cultivars, on the basis of the 13 synonymy groups proposed by De Mattia et al. (2007). In parentheses the number of seeds.

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G11	Nr	NrA	VrE	Sn	VrS	Cl	MPt	MT2	MSP	GB	ArG	ArM	GNC	GNS	Vrt	Total
G1	**81.9 (4379)**	2.2 (116)	0.8 (44)	0.6 (31)	0.1 (4)	0.3 (14)	2.5 (132)	1.2 (64)	0.3 (15)	0.5 (26)	0.8 (44)	0.2 (10)	-	0.7 (39)	1.3 (70)	0.02 (1)	0.2 (12)	0.3 (14)	0.04 (2)	1.3 (67)	0.7 (36)	1.7 (93)	0.6 (29)	1.4 (74)	0.6 (31)	100 (5349)
G2	8.8 (290)	**53.4 (1759)**	4.6 (151)	1.9 (61)	7.8 (257)	0.3 (9)	0.4 (14)	3.8 (126)	-	0.1 (2)	0.9 (30)	1.1 (36)	1.5 (51)	0.9 (29)	1.2 (39)	0.1 (4)	0.5 (15)	0.5 (16)	-	2.6 (85)	0.2 (7)	3.8 (124)	1.2 (40)	4.1 (136)	0.4 (13)	100 (3294)
G3	14.6 (175)	20.2 (242)	**43.1 (515)**	1.2 (14)	3.3 (39)	1.1 (13)	2.1 (25)	1.6 (19)	-	-	0.1 (1)	0.6 (7)	0.5 (6)	1.2 (14)	0.3 (4)	0.0 (0)	0.8 (10)	1.7 (20)	0.2 (1)	0.2 (2)	0.1 (1)	0.2 (2)	3.1 (37)	2.4 (29)	1.7 (20)	100 (1196)
G4	4.0 (72)	2.4 (43)	0.4 (8)	**69.5 (1250)**	0.7 (12)	1.6 (29)	0.7 (12)	0.3 (5)	1.3 (23)	0.7 (13)	3.8 (68)	2.7 (48)	0.3 (6)	0.9 (16)	0.9 (17)	1.0 (18)	0.8 (15)	1.2 (22)	1.9 (35)	1.2 (21)	0.2 (4)	-	1.3 (23)	1.7 (31)	0.4 (8)	100 (1799)
G5	0.4 (10)	4.8 (114)	2.2 (52)	0.3 (7)	**76.9 (1839)**	0.5 (13)	0.1 (3)	0.0 (1)	-	-	0.3 (6)	0.2 (5)	9.4 (225)	0.1 (2)	0.5 (13)	0.8 (18)	0.8 (19)	0.5 (11)	-	1.0 (25)	0.0 (1)	0.1 (3)	0.2 (4)	0.5 (13)	0.3 (6)	100 (2390)
G6	0.5 (8)	1.4 (24)	1.4 (24)	1.9 (34)	1.8 (32)	**69.0 (1226)**	7.4 (132)	-	2.0 (36)	0.9 (16)	1.0 (18)	0.2 (4)	0.1 (2)	1.8 (32)	1.4 (24)	0.8 (14)	1.2 (22)	0.8 (14)	1.0 (18)	-	-	0.1 (2)	3.3 (58)	1.8 (32)	0.2 (4)	100 (1766)
G7	13.5 (243)	1.3 (24)	1.4 (26)	0.1 (1)	0.3 (5)	4.0 (72)	**62.2 (1121)**	-	3.1 (55)	0.2 (4)	0.1 (1)	0.1 (2)	-	5.6 (101)	2.2 (39)	0.2 (4)	0.6 (10)	0.8 (15)	1.6 (29)	-	0.1 (1)	-	1.8 (33)	0.3 (6)	0.6 (11)	100 (1803)
G8	7.9 (95)	4.4 (53)	0.3 (4)	0.1 (1)	-	0.2 (2)	0.1 (1)	**80.4 (962)**	0.1 (1)	0.5 (6)	-	-	-	0.3 (3)	1.2 (14)	-	0.4 (5)	-	-	-	1.4 (17)	1.5 (18)	-	0.3 (4)	0.8 (10)	100 (1196)
G9	7.2 (43)	-	0.2 (1)	1.2 (7)	-	1.7 (10)	3.2 (19)	0.2 (1)	**45.6 (272)**	15.2 (91)	-	-	-	-	-	0.7 (4)	0.5 (3)	2.5 (12)	19.1 (114)	-	-	-	1.3 (8)	0.7 (4)	1.3 (8)	100 (597)
G11	5.2 (31)	0.3 (2)	0.2 (1)	3.9 (23)	-	1.5 (9)	0.8 (5)	1.7 (10)	11.1 (66)	**48.5 (289)**	-	-	-	0.2 (1)	-	-	0.2 (1)	0.2 (1)	23.2 (138)	-	-	-	1.0 (6)	0.5 (3)	1.7 (10)	100 (596)
Nr	4.1 (36)	5.2 (46)	1.6 (14)	27.9 (248)	1.7 (15)	1.9 (17)	0.5 (4)	0.9 (8)	0.9 (8)	2.4 (21)	**35.2 (313)**	1.2 (11)	0.2 (2)	1.8 (16)	0.1 (1)	0.6 (5)	0.8 (7)	0.7 (6)	4.6 (41)	4.6 (41)	0.6 (5)	1.0 (9)	0.6 (5)	1.0 (9)	-	100 (888)
NrA	7.0 (42)	4.7 (28)	2.3 (14)	48.3 (289)	2.7 (16)	1.0 (6)	0.8 (5)	0.3 (2)	1.0 (6)	-	6.0 (36)	**12.2 (73)**	0.2 (1)	0.7 (4)	2.3 (14)	0.3 (2)	1.7 (10)	2.3 (14)	1.5 (9)	0.7 (4)	0.3 (2)	0.2 (1)	1.0 (6)	2.0 (12)	0.3 (2)	100 (598)
VrE	-	3.9 (24)	0.5 (3)	1.5 (9)	36.0 (219)	0.5 (3)	-	-	-	-	-	0.2 (1)	**56.1 (341)**	-	0.2 (1)	0.5 (3)	-	-	-	0.3 (2)	-	-	0.3 (2)	-	-	100 (608)
Sn	3.5 (42)	0.9 (11)	1.1 (13)	-	0.3 (3)	5.2 (62)	18.0 (215)	0.6 (7)	0.9 (11)	0.7 (8)	0.6 (7)	-	-	**51.7 (617)**	0.5 (6)	0.1 (1)	0.2 (2)	0.2 (2)	0.4 (5)	-	1.6 (19)	1.3 (16)	7.6 (91)	3.2 (38)	1.4 (17)	100 (1193)
VrS	11.3 (119)	5.0 (52)	1.8 (19)	3.1 (33)	1.0 (10)	2.3 (24)	5.8 (61)	0.5 (5)	0.6 (6)	0.3 (3)	0.3 (3)	0.8 (8)	0.3 (3)	1.0 (10)	**53.3 (559)**	0.4 (4)	0.7 (7)	0.3 (3)	-	1.5 (16)	0.4 (4)	2.4 (25)	2.5 (26)	2.9 (30)	1.8 (19)	100 (595)
Cl	0.5 (3)	0.5 (3)	0.2 (1)	7.0 (42)	1.5 (9)	1.8 (11)	2.3 (14)	0.2 (1)	1.3 (8)	-	-	0.2 (1)	0.3 (2)	0.2 (1)	0.7 (4)	**57.1 (343)**	13.6 (82)	12.1 (73)	-	0.2 (1)	-	-	-	0.2 (1)	0.2 (1)	100 (601)
MPt	2.8 (17)	3.2 (19)	5.2 (31)	6.5 (39)	3.2 (19)	2.3 (14)	7.7 (46)	0.3 (2)	0.5 (3)	0.5 (3)	0.5 (3)	0.7 (4)	-	0.5 (3)	1.5 (9)	10.8 (65)	**33.5 (201)**	13.5 (81)	-	0.2 (1)	0.3 (2)	-	1.2 (7)	3.0 (18)	2.2 (13)	100 (600)
MT2	2.7 (16)	0.2 (1)	2.8 (17)	5.2 (31)	2.5 (15)	1.2 (7)	2.3 (14)	-	1.8 (11)	0.3 (2)	0.2 (1)	0.2 (1)	0.2 (1)	-	-	5.0 (30)	11.5 (69)	**61.3 (368)**	0.8 (5)	0.2 (1)	-	-	0.3 (2)	0.3 (2)	1.0 (6)	100 (600)
MSP	2.0 (12)	0.3 (2)	0.2 (1)	6.5 (39)	-	0.7 (4)	2.7 (16)	-	12.0 (72)	24.7 (148)	-	0.7 (4)	-	0.2 (1)	-	0.5 (3)	0.7 (4)	0.2 (1)	**47.8 (287)**	-	0.2 (1)	-	0.3 (2)	0.3 (2)	0.2 (1)	100 (600)
GB	13.1 (137)	20.1 (211)	1.1 (12)	4.8 (50)	5.2 (55)	1.0 (10)	0.4 (4)	0.1 (1)	-	0.1 (1)	2.6 (27)	0.5 (5)	1.0 (10)	0.5 (5)	1.1 (12)	0.3 (3)	0.1 (1)	0.1 (1)	0.2 (2)	**39.0 (409)**	-	2.2 (23)	0.8 (8)	5.8 (61)	0.1 (1)	100 (1049)
ArG	8.5 (51)	0.8 (5)	0.2 (1)	0.2 (1)	-	-	0.2 (1)	5.5 (33)	-	-	-	-	-	0.5 (3)	2.2 (13)	-	0.3 (2)	-	0.3 (2)	-	**76.9 (460)**	4.0 (24)	-	0.2 (1)	0.2 (1)	100 (598)
ArM	18.0 (214)	6.3 (75)	-	0.2 (2)	0.3 (3)	0.3 (4)	0.2 (2)	8.6 (102)	-	-	0.3 (3)	-	-	1.9 (23)	1.2 (14)	-	-	-	-	1.1 (13)	6.6 (79)	**53.8 (641)**	0.9 (11)	0.3 (4)	0.2 (2)	100 (1192)
GNC	7.7 (92)	3.8 (45)	1.5 (18)	0.5 (6)	1.3 (16)	2.4 (29)	2.9 (35)	0.6 (7)	1.8 (21)	0.5 (6)	0.1 (1)	0.3 (3)	-	4.2 (50)	4.2 (50)	0.6 (7)	0.6 (7)	0.5 (6)	0.7 (8)	1.2 (14)	0.2 (2)	0.7 (8)	**43.4 (520)**	11.3 (135)	9.4 (113)	100 (1199)
GNS	8.2 (98)	7.6 (91)	4.2 (50)	1.3 (15)	0.8 (9)	2.8 (34)	1.9 (23)	1.3 (15)	0.2 (2)	1.3 (15)	0.9 (11)	0.3 (3)	-	4.3 (51)	1.4 (17)	0.1 (1)	0.7 (8)	0.8 (9)	0.3 (4)	0.4 (5)	0.1 (1)	0.3 (4)	7.3 (87)	**44.7 (536)**	9.3 (111)	100 (1200)
Vrt	8.4 (50)	6.2 (37)	6.2 (37)	0.2 (1)	0.7 (4)	1.0 (6)	7.7 (46)	4.5 (27)	1.8 (11)	2.5 (15)	-	0.2 (1)	-	2.3 (14)	2.0 (12)	-	1.3 (8)	0.5 (3)	0.7 (4)	0.7 (4)	-	-	7.9 (47)	18.1 (108)	**27.3 (163)**	100 (598)
Overall																										**59.7 (32569)**

Table 4 – Statistical comparisons among the analysed black berry cultivars, on the basis of the morpho-colorimetical features. In parentheses the number of seeds.

	Cn	FlG	GNC	Mz	NrB	PsC	Vrt	GNS	ME	MSl	MSr	NrT	NPS	NPd	NPt	NPl	PsO	PrN	Total
Cn	**84.3 (1498)**	0.2 (4)	4.4 (78)	3.5 (62)	-	-	2.5 (44)	1.8 (32)	0.2 (4)	0.1 (2)	0.8 (14)	-	-	1.0 (18)	0.3 (6)	0.1 (2)	-	0.7 (12)	100.0 (1776)
FlG	0.8 (5)	**42.0 (250)**	0.2 (1)	5.2 (31)	2.7 (16)	18.0 (107)	0.5 (3)	0.5 (3)	0.2 (1)	1.3 (8)	1.8 (11)	1.7 (10)	8.9 (53)	1.3 (8)	6.2 (37)	3.2 (19)	4.7 (28)	0.7 (4)	100.0 (595)
GNC	3.6 (43)	0.7 (8)	**54.8 (657)**	9.3 (111)	0.5 (6)	3.4 (41)	12.8 (153)	9.4 (113)	-	0.7 (8)	1.1 (13)	0.5 (6)	0.4 (5)	0.6 (7)	1.0 (12)	0.2 (2)	0.1 (1)	1.1 (13)	100.0 (1199)
Mz	0.6 (12)	1.4 (30)	0.9 (18)	**71.7 (1504)**	2.3 (48)	3.1 (64)	6.0 (126)	0.6 (12)	0.5 (10)	2.8 (58)	3.4 (72)	0.1 (2)	0.8 (16)	4.1 (86)	0.6 (12)	0.8 (16)	0.5 (10)	0.1 (2)	100.0 (2098)
NrB	0.5 (3)	1.5 (9)	-	0.2 (1)	**65.5 (393)**	0.7 (4)	0.8 (5)	1.2 (7)	5.5 (33)	1.5 (9)	1.0 (6)	1.2 (7)	1.0 (6)	0.2 (1)	-	17.5 (105)	0.8 (5)	1.0 (6)	100.0 (600)
PsC	0.3 (6)	6.3 (112)	1.7 (30)	5.7 (100)	0.2 (4)	**60.6 (1070)**	2.9 (52)	0.7 (12)	0.8 (14)	2.0 (36)	0.9 (16)	3.1 (54)	3.1 (54)	0.5 (8)	3.7 (66)	1.1 (20)	3.9 (68)	2.5 (44)	100.0 (1766)
Vrt	2.8 (34)	0.2 (2)	9.3 (112)	11.7 (140)	1.3 (16)	1.8 (22)	**55.2 (662)**	11.5 (138)	-	0.6 (7)	1.2 (14)	0.6 (7)	0.3 (4)	1.3 (16)	1.0 (12)	0.2 (2)	0.2 (2)	0.8 (10)	100.0 (1200)
GNS	2.0 (12)	0.5 (3)	11.5 (69)	6.7 (40)	2.8 (17)	1.7 (10)	27.1 (162)	**38.1 (228)**	-	0.5 (3)	2.2 (13)	-	0.2 (1)	3.3 (20)	0.5 (3)	1.8 (11)	0.3 (2)	0.7 (4)	100.0 (598)
ME	-	-	-	1.8 (11)	1.8 (11)	5.3 (32)	0.2 (1)	-	**79.8 (479)**	8.2 (49)	1.2 (7)	-	0.3 (2)	-	1.2 (7)	-	0.2 (1)	-	100.0 (600)
MSl	-	5.2 (31)	1.0 (6)	9.2 (55)	1.0 (6)	5.7 (34)	2.2 (13)	0.7 (4)	7.6 (45)	**56.5 (337)**	0.3 (2)	0.2 (1)	0.3 (2)	2.5 (15)	0.5 (3)	6.7 (40)	0.3 (2)	-	100.0 (596)
MSr	1.2 (7)	1.0 (6)	0.2 (1)	13.7 (82)	1.0 (6)	0.2 (1)	5.0 (30)	3.7 (22)	3.3 (20)	0.7 (4)	**62.8 (377)**	0.2 (1)	0.3 (2)	1.2 (7)	4.0 (24)	-	1.7 (10)	-	100.0 (600)
NrT	1.2 (7)	4.3 (26)	0.5 (3)	1.2 (7)	0.8 (5)	26.5 (159)	0.2 (1)	2.8 (17)	-	0.2 (1)	1.3 (8)	**25.7 (154)**	6.5 (39)	0.7 (4)	10.5 (63)	0.3 (2)	6.0 (36)	11.2 (67)	100.0 (599)
NPS	0.2 (1)	11.5 (69)	-	2.5 (15)	0.7 (4)	19.5 (117)	1.0 (6)	0.5 (3)	-	0.5 (3)	-	3.8 (23)	**40.1 (240)**	0.8 (5)	4.5 (27)	0.8 (5)	9.2 (55)	4.3 (26)	100.0 (599)
NPd	1.2 (7)	1.7 (10)	4.0 (24)	19.3 (115)	0.3 (2)	1.8 (11)	5.9 (35)	1.2 (7)	-	1.7 (10)	0.7 (4)	-	1.7 (10)	**58.6 (349)**	0.7 (4)	1.3 (8)	-	-	100.0 (596)
NPt	1.0 (6)	5.2 (31)	-	1.8 (11)	-	10.9 (65)	3.5 (21)	2.2 (13)	0.2 (1)	-	2.4 (14)	8.2 (49)	1.7 (10)	-	**51.8 (308)**	0.3 (2)	3.5 (21)	7.2 (43)	100.0 (595)
NPl	-	5.0 (30)	-	0.8 (5)	22.0 (131)	1.0 (6)	-	1.3 (8)	1.5 (9)	3.0 (18)	-	0.8 (5)	1.7 (10)	1.0 (6)	-	**60.7 (362)**	0.7 (4)	0.3 (2)	100.0 (596)
PsO	0.7 (4)	8.5 (51)	-	2.5 (15)	0.5 (3)	23.0 (138)	1.5 (9)	1.3 (8)	0.2 (1)	0.3 (2)	1.7 (10)	8.2 (49)	10.5 (63)	-	11.0 (66)	0.3 (2)	**25.4 (152)**	4.3 (26)	100.0 (599)
PrN	0.5 (3)	7.0 (42)	1.8 (11)	0.5 (3)	0.2 (1)	10.6 (63)	1.3 (8)	2.3 (14)	-	0.2 (1)	0.3 (2)	9.2 (55)	6.7 (40)	0.2 (1)	5.5 (33)	-	6.5 (39)	**47.0 (280)**	100.0 (596)
Overall																			**58.8 (15808)**

Table 5. Statistical comparisons among the analysed white berry cultivars, on the basis of the morpho-colorimetical features. In parentheses the number of seeds.

	ArB	ArG	ArM	CnT	Cl	GB	MPt	Nr	Sn	Vr	VrR	VrS	MLd	MT1	MT2	MSP	NrA	Nrt	Nrd	NRR	VrE	CnO	Total
ArB	**68.5 (411)**	-	-	0.2 (1)	0.7 (4)	-	3.2 (19)	-	-	7.8 (47)	6.7 (40)	1.0 (6)	-	-	0.8 (5)	-	1.3 (8)	0.5 (3)	0.3 (2)	0.2 (1)	8.8 (53)	-	100.0 (600)
ArG	0.3 (2)	**88.3 (528)**	5.7 (34)	0.5 (3)	-	0.2 (1)	0.5 (3)	0.3 (2)	0.8 (5)	-	-	2.3 (14)	-	-	-	0.5 (3)	-	-	0.3 (2)	0.2 (1)	-	-	100.0 (598)
ArM	0.3 (4)	9.4 (112)	**73.9 (881)**	1.1 (13)	-	4.4 (53)	-	1.0 (12)	5.2 (62)	0.5 (6)	-	3.7 (44)	0.2 (2)	0.1 (1)	-	-	-	-	0.1 (1)	-	-	0.1 (1)	100.0 (1192)
CnT	0.2 (2)	0.3 (4)	-	**73.2 (878)**	0.3 (4)	0.2 (2)	2.2 (26)	-	9.3 (112)	0.2 (2)	-	5.3 (64)	1.0 (12)	0.8 (10)	0.5 (6)	1.3 (16)	-	-	-	-	-	5.2 (62)	100.0 (1200)
Cl	0.5 (3)	-	-	0.7 (4)	**63.6 (382)**	0.2 (1)	15.6 (94)	0.2 (1)	-	0.2 (1)	0.3 (2)	0.2 (1)	0.2 (1)	1.2 (7)	11.1 (67)	-	1.7 (10)	0.7 (4)	1.3 (8)	1.3 (8)	0.5 (3)	0.7 (4)	100.0 (601)
GB	0.4 (4)	-	3.8 (40)	0.9 (9)	0.2 (2)	**69.7 (731)**	1.3 (14)	5.9 (62)	1.6 (17)	4.7 (49)	0.4 (4)	3.2 (34)	-	-	0.4 (4)	0.3 (3)	1.3 (14)	2.0 (21)	1.5 (16)	0.2 (2)	2.2 (23)	-	100.0 (1049)
MPt	1.5 (9)	0.7 (4)	-	7.5 (45)	11.0 (66)	1.2 (7)	**47.5 (285)**	1.3 (8)	0.7 (4)	0.8 (5)	0.2 (1)	2.0 (12)	0.8 (5)	0.3 (2)	15.3 (92)	-	3.0 (18)	2.5 (15)	1.3 (8)	0.3 (2)	0.8 (5)	1.2 (7)	100.0 (600)
Nr	2.1 (19)	0.5 (4)	2.1 (19)	0.5 (4)	0.6 (5)	6.8 (60)	1.6 (14)	**49.7 (441)**	2.9 (26)	1.1 (10)	0.1 (1)	0.6 (5)	2.9 (26)	0.8 (7)	0.8 (7)	3.7 (33)	3.2 (28)	9.3 (83)	5.0 (44)	5.4 (48)	0.2 (2)	0.2 (2)	100.0 (888)
Sn	0.2 (2)	1.4 (17)	1.1 (13)	11.1 (133)	0.1 (1)	0.4 (5)	0.5 (6)	1.3 (15)	**70.5 (841)**	0.3 (4)	-	1.0 (12)	1.5 (18)	1.8 (22)	0.6 (7)	0.3 (3)	0.2 (2)	0.2 (2)	0.1 (1)	-	-	7.5 (89)	100.0 (1193)
Vr	5.4 (64)	-	0.4 (5)	0.2 (2)	1.2 (14)	3.5 (42)	2.1 (25)	0.3 (3)	0.3 (3)	**63.6 (759)**	9.7 (116)	0.9 (11)	-	-	0.7 (8)	-	0.6 (7)	0.1 (1)	-	0.1 (1)	11.1 (132)	-	100.0 (1193)
VrR	14.1 (84)	-	-	0.2 (1)	1.3 (8)	0.7 4()	2.7 (16)	0.3 (2)	-	17.8 (106)	**42.7 (255)**	0.3 (2)	-	-	0.8 (5)	-	-	0.2 (1)	-	-	18.9 (113)	-	100.0 (597)
VrS	0.9 (9)	0.6 (6)	2.8 (29)	6.0 (63)	0.5 (5)	3.3 (35)	2.8 (29)	0.8 (8)	2.6 (27)	0.4 (4)	0.2 (2)	**69.6 (730)**	0.7 (7)	1.0 (11)	1.0 (10)	0.2 (2)	1.5 (16)	1.0 (10)	1.1 (12)	0.8 (8)	0.3 (3)	2.2 (23)	100.0 (1049)
MLd	-	0.3 (2)	-	2.0 (12)	0.2 (1)	0.2 (1)	1.0 (6)	0.3 (2)	0.8 (5)	-	-	0.3 (2)	**56.0 (334)**	11.4 (68)	0.2 (1)	23.3 (139)	0.2 (1)	2.2 (13)	1.0 (6)	0.2 (1)	-	0.3 (2)	100.0 (596)
MT1	-	-	-	2.3 (14)	0.5 (3)	-	2.2 (13)	0.7 (4)	0.3 (2)	-	-	0.2 (1)	17.8 (106)	**49.9 (298)**	3.2 (19)	19.3 (115)	0.3 (2)	0.2 (1)	-	0.3 (2)	-	2.8 (17)	100.0 (597)
MT2	1.2 (7)	-	-	0.7 (4)	7.0 (42)	-	12.3 (74)	0.3 (2)	0.3 (2)	0.5 (3)	-	0.3 (2)	0.2 (1)	1.5 (9)	**69.0 (414)**	0.3 (2)	1.8 (11)	0.3 (2)	1.2 (7)	0.7 (4)	-	2.3 (14)	100.0 (600)
MSP	0.2 (1)	-	-	0.8 (5)	0.7 (4)	-	1.0 (6)	0.2 (1)	0.2 (1)	-	-	-	24.5 (147)	11.8 (71)	0.5 (3)	**53.0 (318)**	2.2 (13)	1.3 (8)	0.7 (4)	2.2 (13)	-	0.8 (5)	100.0 (600)
NrA	2.7 (16)	0.3 (2)	0.3 (2)	1.0 (6)	0.8 (5)	2.8 (17)	5.9 (35)	5.7 (34)	1.3 (8)	0.8 (5)	0.2 (1)	4.2 (25)	0.2 (1)	1.0 (6)	3.3 (20)	1.7 (10)	**31.9 (191)**	11.7 (70)	12.2 (73)	11.2 (67)	0.7 (4)	-	100.0 (598)
Nrt	1.7 (10)	-	-	0.3 (2)	1.3 (8)	1.3 (8)	2.0 (12)	6.3 (38)	2.0 (12)	0.3 (2)	-	4.0 (24)	1.3 (8)	1.0 (6)	1.0 (6)	0.3 (2)	10.0 (60)	**49.7 (298)**	9.3 (56)	8.0 (48)	-	-	100.0 (600)
Nrd	0.2 (1)	0.3 (2)	0.5 (3)	0.2 (1)	1.3 (8)	1.8 (11)	1.7 (10)	6.0 (36)	0.2 (1)	-	-	2.7 (16)	1.2 (7)	0.5 (3)	2.3 (14)	3.3 (20)	10.7 (64)	10.2 (61)	**40.5 (243)**	15.5 (93)	1.0 (6)	-	100.0 (600)
NRR	0.3 (2)	1.2 (7)	-	0.2 (1)	1.7 (10)	3.2 (19)	2.5 (15)	5.3 (32)	1.8 (11)	-	0.3 (2)	2.2 (13)	1.8 (11)	2.7 (16)	3.3 (20)	4.0 (24)	12.4 (74)	7.3 (44)	22.7 (136)	**25.5 (153)**	0.8 (5)	0.7 (4)	100.0 (599)
VrE	7.6 (46)	-	-	-	0.5 (3)	1.0 (6)	0.7 (4)	-	-	8.6 (52)	11.2 (68)	0.7 (4)	-	-	0.2 (1)	-	1.2 (7)	1.0 (6)	0.2 (1)	-	**67.4 (410)**	-	100.0 (608)
CnO	0.5 (3)	0.8 (5)	0.2 (1)	26.0 (157)	0.5 (3)	-	3.2 (19)	-	8.5 (51)	0.3 (2)	-	1.0 (6)	0.7 (4)	6.3 (38)	2.8 (17)	0.8 (5)	0.7 (4)	0.2 (1)	0.2 (1)	-	-	**47.4 (286)**	100.0 (603)
Overall																							**60.1 (16761)**

Tables 4 and 5 show the results of the comparisons among the analysed black berry and white berry cultivars, respectively. In the first case, an overall percentage of correct discrimination of 58.8% was reached, while white berry cultivars were correctly identified in the 60.1% of the cases.

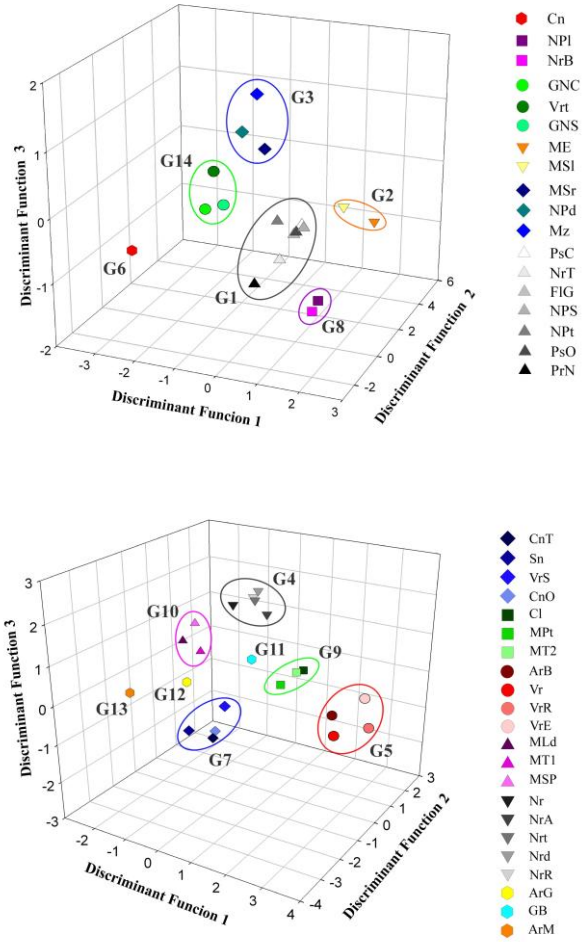

Figure 2 and Figure 3

Figures 2 and 3 show the graphical representations of the achieved groupings from the morpho-colorimetric comparisons among the black berry and white berry cultivars, respectively. Although the 3D plots were drown using only three of the about 20 discriminant functions implemented to distinguish the varieties in both the cases, these graphical representations well highlight the phenotypical differences among the membership groups, organizing little clearly identifiable groups. The three discriminant functions used to draw the

plots were able to explain the 66.3% and the 70.7% of the whole statistical variability for the black berry and white berry cultivars comparisons, respectively (data not shown).

Following the suggestions of the implemented classifiers, on the basis of the achieved results by the two discriminant statistical elaborations, two further discrimination analysis were carried out, moving some of cultivars from a group to another and hypothesizing new synonymy groups. It was possible to identify six black berry cultivar synonymy groups (Tab. 6) and eight white berry cultivar synonymy groups (Tab. 7).

Table 6. Percentages of correct classification for the new hypothetical synonymy black cultivar groups. In parentheses the number of seeds.

	G1	G2	G3	G6	G8	G14	Total
G1	**88.9 (4757)**	0.8 (41)	4.5 (243)	0.4 (21)	1.4 (74)	4.0 (213)	100.0 (5349)
G2	12.3 (147)	**68.6 (820)**	9.9 (119)	-	5.5 (66)	3.7 (44)	100.0 (1196)
G3	8.7 (286)	3.2 (104)	**77.4 (2551)**	0.7 (23)	1.7 (56)	8.3 (274)	100.0 (3294)
G6	1.4 (24)	0.2 (4)	5.7 (102)	**82.3 (1462)**	0.1 (2)	10.2 (182)	100.0 (1776)
G8	8.8 (105)	5.9 (70)	1.7 (20)	0.2 (2)	**81.3 (972)**	2.3 (27)	100.0 (1196)
G14	7.2 (216)	0.2 (5)	12.9 (388)	3.1 (92)	1.6 (49)	**75.0 (2247)**	100.0 (2997)
Overall							**81.0 (15808)**

Table 7. Percentages of correct classification for the new hypothetical synonymy white cultivar groups. In parentheses the number of seeds.

	G4	G5	G7	G9	G10	G11	G12	G13	Total
G4	**80.4 (2640)**	1.9 (64)	4.9 (162)	4.5 148()	3.8 (125)	3.3 (108)	0.5 (15)	0.7 (23)	100.0 (3285)
G5	1.7 (50)	**90.8 (2722)**	1.8 (55)	3.3 (100)	-	2.1 (63)	-	0.3 (8)	100.0 (2998)
G7	2.6 (105)	1.1 (43)	**85.2 (3446)**	2.5 (102)	4.1 (164)	1.7 (67)	1.0 (42)	1.9 (76)	100.0 (4045)
G9	8.2 (148)	2.3 (42)	8.9 (160)	**79.2 (1427)**	1.2 (21)	0.1 (2)	0.1 (1)	-	100.0 (1801)
G10	5.4 (97)	0.1 (1)	4.4 (79)	2.6 46()	**87.5 (1569)**	-	0.1 (1)	-	100.0 (1793)
G11	14.6 (153)	7.5 (79)	7.4 (78)	1.5 (16)	0.3 (3)	**64.3 (675)**	0.1 (1)	4.2 (44)	100.0 (1049)
G12	2.0 (12)	-	4.5 (27)	-	0.3 (2)	0.3 (2)	**85.8 (513)**	7.0 (42)	100.0 (598)
G13	0.9 (11)	0.9 (11)	9.3 (111)	-	0.3 (4)	4.3 (51)	10.4 (124)	**73.8 (880)**	100.0 (1192)
Overall									**82.80 (16761)**

The black berry cultivar Manzesu (Mz) was moved from the group 2 (G2) proposed by De Mattia et al. (2007) where it was considered synonym of Monica di Escalaplano (ME) and Monica di Seulo (MSl), to the G3 together with Monica di Sorgono (MSr) and Nieddu Mannu di Padria (NPd). The displacement of this black berry cultivar, brought to the increment of the percentages of correct identification of the two groups from 53.4% (Tab. 3) to 68.6% (Tab. 6) and 43.1% (Tab. 3) to 77.4% (Tab. 6) for G2 and G3 respectively (Fig. 2). According to De Mattia et al. (2007), considering the wide diffusion of the Monica cultivars in Sardinia, the presence of the variety known as Monica di Sorgono (MSr) into the G3 rather than into the G2 together with the other Monica cultivars, suggests the possibility that a wrong name was historically attributed to this cultivar. Moreover, this modification allows to suppose that probably the cultivar named Manzesu (Mz) should be the same variety of those currently known as Monica di Sorgono (MSr) and Nieddu Mannu di Padria (NPd) rather than of Monica di Escalaplano (ME) and Monica di Seulo (MSl) as reported by De Mattia et al. (2007). This supposition is also graphically supported by the spatial distance of the barycentre discriminant scores of these cultivars (Fig. 2).

Moreover, the white berry cultivars Nuragus (Nr) and Nuragus Arrubiu (NrA), considered as independent by De Mattia et al. (2007), were moved into the G4 together with Nuragus Moscatello (Nrt), Nuragus Rosso Rompizzolla (NrR) and Nuragus Moscadeddu (Nrd). Similarly to the previous case, this modification given a rising of the performance from 69.5% (Tab. 3) to 80.4% (Tab. 7) for this synonymy group (Fig. 3).

Also the independent white berry cultivar Vernaccia di Escalaplano (VrE) was moved into the G5 together with Vernaccia di S. Rosalia (VrR), Vernaccia (Vr) and Aregu Biancu (ArB), producing the same effect on this synonymy group which percentage of correct identification increased from 76.9% (Tab. 3) to 90.8% (Tab. 7), allowing to advance the hypothesis that these four grapevine varieties are the same cultivar (Fig. 3).

The G7, constituted by Cannonau bianco di Triei (CnT) and Cannonau bianco di Oliena (CnO) was enriched with Vernaccia di Solarussa (VrS) and Sinnidanu (Sn), both considered as independent by De Mattia et al. (2007). This arrangement shown an improvement of the discrimination performance, bringing to the increment of the percentages of correct identification of this group from 62.2% (Tab. 3) to 85.2% (Tab. 7).

Another interesting case of potential synonymy is represented by Moscato di Tempio 1 (MT1), Moscato di Lodine (MLd) and Moscato Su Pinu (MSP) (Fig. 3). Although these three cultivars were considered different by De Mattia et al. (2007) even if genetically close one to the other, here they are included in a new synonymy group (here labelled as G10) on the basis of germplasm phenotypic characters. This group shown a correct identification percentage of equal to 87.5% (Tab. 7).

Similar considerations for the white berry cultivars Culupuntu (Cl), Moscato di Tempio 2 (MT2) and Moscato di Pattada (MPt) (here labelled as G9). The 79.2% of correct identification obtained for this group (Tab. 7), as well as the graphical representation of the spatial distance of the discriminant scores (Fig. 3) should confirm the advanced supposition.

The same observations were done for the independent black berry cultivars known as Vertudi (Vrt), Gregu Nieddu del Campidano (GNC) and Gregu Nieddu di Serramanna (GNS) (Tabs. 3 and 4). The new synonymy G14 was carried out, reaching 75.0% of correct identification (Tab. 6) and confirming the possibility that these three cultivars are the same (Fig. 2).

Finally, according to the findings of De Mattia et al. (2007), the white berry cultivars Gregu Bianco (GB), Aregu Giallo (ArG) and Argu Mannu (ArM) (here labelled as G11, G12 and G13, respectively) seems to be phenotypically and genotipically different when compared with the other studied cultivars, showing percentages of correct discrimination included between 64.3% and 85.8% (Tab. 7 and Fig. 3).

Considering the new synonymy groupings here proposed, the overall percentages of correct identification risen up to 81.0% for the six black berry cultivar groups (Tab. 6) and 82.8% for the eight white berry cultivar groups (Tab. 7).

Table 8 shows the comparison between the synonymy groups proposed by De Mattia et al. (2007) on the basis molecular data and the new hypothetical synonymy groups achieved on the basis of morpho-colorimetrical data. It is possible to notice that six of the 14 groups remained unchanged (G1, G6, G8, G11, G12 and G13), five of the studied varieties were unified to groups proposed by De Mattia et al. (2007) (Nuragus and Nuragus Arrubiu in G4, Vernaccia di Escalaplano in G5 and Sinnidanu and Vernaccia di Solarussa in G7), two groups were modified (G2 and G3) and three new groups were formed (G9, G10 and G14).

Table 8. Comparison between synonymy group notations proposed by De Mattia et al. (2007) on the basis molecular data and the new hypothetical synonymy groups achieved on the basis of morpho-colorimetrical data. In bold the modifications.

Grape variety	Previous synonymy group according to De Mattia et al. (2007)	New synonymy group
Falso Gregu	G1	CG
Nera Tomentosa	G1	CG
Nieddu Mannu di Pattada	G1	CG
Nieddu Pedra Serra	G1	CG
Pascale di Caglairi	G1	CG
Pascale di Oliena	G1	CG
Primidivu Nieddu	G1	CG
Monica di Escalaplano	G2	CG
Monica di Seulo	G2	CG
Manzesu	G2	G3
Monica di Sorgono	G3	CG
Nieddu Mannu di Padria	G3	CG
Nuragus Moscadeddu	G4	CG
Nuragus Moscatello	G4	CG
Nuragus Rosso Rompizzolla	G4	CG
Nuragus	IG	G4
Nuragus Arrubiu	IG	G4
Aregu Biancu	G5	CG
Vernaccia	G5	CG
Vernaccia di S. Rosalia	G5	CG
Vernaccia di Escalaplano	IG	G5
Cannonau	G6	CG
Cannonau Bianco di Oliena	G7	CG
Cannonau Bianco di Trieri	G7	CG
Sinnidanu	IG	G7
Vernaccia di Solarussa	IG	G7
Nero di Bosa	G8	CG
Nieddu Polchino	G8	CG
Culupuntu	IG	G9
Moscato di Pattada	IG	G9
Moscato di Tempio 2	IG	G9
Moscato di Tempio 1	G9	G10
Moscato di Lodine	G11	G10
Moscato Su Pinu	IG	G10
Gregu Bianco	IG	G11
Aregu Giallo	IG	G12
Argu Mannu	IG	G13
Gregu Nieddu del Campidano	IG	G14
Gregu Nieddu di Serramanna	IG	G14
Vertudi	IG	G14
CG = confirmed group; IG = indipendent group		

5.5. Conclusions

As discussed above, Sardinia is characterized by a huge number of cultivars (Grassi et al. 2003, 2008, Lovicu et al. 2010). Some of these surely derive from different breeding events involving both local and out-coming material, as well as both domesticated and wild grapes, but despite the large and complex grapevine survey, a lot of Sardinian varieties simply are the product of linguistic distorting due to the wide heterogeneity historic-cultural of the island. This phenomena generated a great assortment of grape names, that, together with the huge real number of cultivars, is the cause of the incredible current grapevine Sardinian panorama (De Mattia et al. 2007).

Many times molecular methods proved to be able to screen biodiversity genotyping and comparing different plant cultivars, allowing direct inferences about genetic diversity and interrelationships among organisms at the DNA level without the confounding effects of the environment and/or faulty pedigree records. Nevertheless, considering that the various molecular marker techniques are based on different principles, the importance to find the right compromise between reliability and ease of analysis, statistical power and confidence of revealing polymorphisms, is extreme, and the found solutions not always satisfactory (Agarwal et al. 2008). Moreover, frequently costs and execution times of these techniques are the undoubting disadvantages (Dreher et al. 2003). Furthermore, it is important to consider a few inherent problems associated with the use of SSR markers. Sometimes, homoplasy is a phenomenon observed when a character present in two species is not derived from a common ancestry but rather, the similarity is a result of convergence, parallelism or reversion, so different copies of a locus are identical in state despite not being identical by descent (Park et al. 2009). Other times, in SSR analyses of a large number of samples from diverse germplasms, a few samples fail to produce PCR products, causing the impossibility to determine whether the absence of PCR products represents true null alleles of the SSR locus

or is due to a failure of the PCR reaction (Park et al. 2009). Finally, depending from genome samples, the molecular analysis of a restricted sequences and few alleles is not enough to discriminate among different cultivars, and the analysed alleles are not always related to varietal characters. For this reason, the potentialities of computer vision as complementary to the molecular methods, were here presented, highlighting how a wide gamma of phenotypic characters can be a valid choice to evaluate differences among cultivars also in a so complex scenario.

In the case of the synonymy study of grapevine cultivars, the 113 measured morpho-colorimetric features of the germplasm resulted enough to achieve a clear discrimination among the synonymy groups, as confirmed by the previous SSR analysis conducted by De Mattia et al. (2007) on the same material. Finally, it could be very interesting to assess the contribute of a supporting ampelographic characterization, in particular using image analysis techniques on grapevine leaves, in the varietal identification, as well as in the synonyms and false attribution evaluations.

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6. Seeds morpho-colorimetric analysis by computer vision: an powerfull tool to predict the grapewines (*Vitis vinifera* L.) cultivars.

6.1. Abstract

The wine heritage in Sardinia consists of 151 cultivars, considerated as local varieties are the product of different events, such as direct domestication of wild grape, crosses between local varieties and the importation of agricultural techniques and cultivars from different ethnic groups that colonised the Island. The remaining varieties can be considered as false attributions (synonyms and/or homonyms) due to the existence of different dialects within the same territory.

Considering the convinceing results achived with the synonymy study of grapevine cultivars conducted on the basis of morphocolorimetric features. The aim of the present study is to use the same seed morpho-colorimetric features and EFDs obtained by image analysis to implement dedicated statistical classifiers able to discriminate among to the studied cultivars of grapewine, also considering specific aspects as grape colour (black or white) and consumption aptitude (table grape or wine grape and table wine, moscat wine or dessert wine). The achieved results seems to be consistent to the geographical distribution of the grapevine cultivar and to the historical and cultural knowledge and are in perfect harmony with previous studies.

6.2. Introduction

Sardinia with a dimension of 24.084 Km^2 is the second largest island in the Mediterranean Sea and due to its geographical position and extremely diversified ecological

condition, hosts an ideal environment for both the growth of wild grape (*V. v. sylvestris*) and grapewine species (*V. v. vinifera*) (Zecca 2010).

During the quaternary glaciations, the island was an area of refuge for the *V. v. sylvestris*, but this species did not play any role in the recolonization of wild grapes in C-Europe because of its distance from the Italian continent (Grassi et al., 2008). Such as insularity condition and the presence of abundant populations of wild grape, allowed the developing processes of independent domestication. Grassi et al. (2003) highlighted how the gene pool of 2 autochthonous cultivars (Bovale Muristellu and Bovale Murru) is similar to wild grapes collected in nearby countryside area of Nuoro, giving evidence that Sardinia is a possible secondary center of domestication of the *V. v. sylvestris*. Phenomena of independent domestication of different autochthonous cultivars of wine have been found in other Mediterranean regions such as Spain and Greece (Arroyo-Garcia, 2002) where more than 70% of autochthonous cultivars seem to descend from Spanish populations of wild grape (Arroyo-Garcia et al., 2006).

The large number of traditionally cultivars considerated as local varieties are the product of different events, such as direct domestication of wild grape, crosses between local varieties and the importation of agricultural techniques and cultivars from different ethnic groups who colonised the Island (De Mattia et al., 2007). A recent study by De Mattia et al. (2009), shows that there isn't genetic relationship between varieties of Vernaccia (or Granaccia) of the Sardinian group with Garnacha Tinta and Garnacha Blanca of the Spanish group, and neither of them was originated from the heritage of wild grape in Sardinia, but through the ancient selection process. Moreover, molecular studies conducted by De Mattia et al. (2009) shown that Cannonau of Sardinia and the Spanish Garnacha Tinta are synonymous.

Currently, the wine heritage in Sardinia consists of 151 cultivars, while the remaining varieties can be considered as false attributions (synonyms and/or homonyms) due to the existence of different dialects within the same territory (Lovicu et al., 2010).

Although Sardinia is characterized by a rich varietal heritage kept and distributed homogeneously throughout the territory, owing to the strong spirit of the old winemarkers (Lovicu, 2007), the major studies on autochthonous cultivars are related to wine varieties.

Many authors (Appelhans et al., 2011; Fawzi, 2011; Rovner and Gyulai, 2007; Bacchetta et al., 2008; 2011; Mattana et al., 2008; Grillo et al., 2010) are agree considering the importance of the biometric features in taxonomic studies to characterize and identify seed lots of wild and cropped plant species, also considering the coming of powerful technologies that allow to measure morphometric and colourimetic parameters in a very accurate and reliable way, overcomeing some limits due to objective evaluations done roughly by specialized technicians.

The recent literature proves how, the computer vision are carring weight also in seed characterization studies. Bacchetta et al. (2008) implemented a database of seed morpho-colorimetric features, measured on digital image captured by a flatbed scanner, of the six most representative families of the Sardinia wild flora (Apiaceae, Boraginaceae, Caryophyllaceae, Cistaceae, Fabaceae and Scrophulariaceae), in order to discriminate among them on the basis of seed images. In a following work, the number of characterized families was incremented (adding Asteraceae, Brassicaceae, Lamiaceae and Poaceae), also including the genus *Juniperus*, suggesting that the applied technology is also reliable for gymnosperms (Grillo et al. 2010). Herridge et al. (2011) used the same method to rapidly analyse the seed size in *Arabidopsis thaliana,* and Grillo et al. (2012) for the seeds discrimination among 27 taxa within the genus *Diplotaxis* DC.

Currently, image analysis techniques are commonly used to characterize seed lots of cropped species. Venora et al. (2007) used morphometric and colorimetric parameters, measured by image analysis to identify and classify different varieties and landraces of *Lens culinaris*, to discriminate among different varieties of *Phaseolus vulgaris* (Venora et al. 2009) and than to distinguish different vetch varieties (Grillo et al, 2011). Similar technologies were

used by Firatligil-Durmus et al. (2010) to study the size properties of lentil seeds, by Dana and Ivo (2008) to analyze the morpho-colorimetric aspects of flax seed lots, and by Smykalova et al. (2011) to study the phenotipical differences among five *Pisum sativum* varieties, and the effect of locality on pea seeds traits.

Computer vision techniques are today applied also to investigate *Vitis* ssp. from many points of view. Tsialtas et al. (2008) used image analysis to study the effect of rootstock and irrigation regime on size and shape of Cabernet-Sauvignon leaves. Peressotti et al. (2010), applied computer vision to quantify grapevine downy mildew sporulation on leaves, requiring just a compact digital camera and the an open source software to analyse the captured images. In another recent paper, Terral et al. (2010) discussed the potentiality of morphometric analysis to compare archaeological seeds od *Vitis*, using the Elliptic Fourier Descriptors (EFD) method. Similarly, Gong et al. (2010) used digital images to analyze the morphometry of same of *Vitis* fossil seeds, identifying three different morphotaxa. In another recent paper, Orrù et al. (2012) used morphological features and Elliptic Fourier Descriptors to characterize archaeological seeds of *Vitis* and to compare them with those of same *V. v. sylvestris* populations and *V. v. vinifera* cultivars.

As for the seeds of any plant species, cropped and wild, grapevine seeds, that also are highly polymorphic, play a crucial role in the taxonomic study within the genus *Vitis* L. (Rivera et al. 2007), as well as in the distribution and domestication processes of the wild grapevine, in many archaeological discoveries (This et al. 2006) and in the study about the identification and grouping of diasporas of *Vitis*.

Considering the convinceing results achived with the synonymy study of grapevine cultivars conducted by Orrù et al. (2012b) un the basis of 113 morpho-colorimetric features compared with the previous SSR analysis conducted by De Mattia et al. (2007) on the same material, the aim of the present study is to use the same seed morpho-colorimetric features and EFDs obtained by image analysis to implement dedicated statistical classifiers able to

discriminate among to the studied cultivars of grapewine, also considering specific aspects as grape colour (black or white) and consumption aptitude (table grape or wine grape and table wine, moscat wine or dessert wine).

6.3. Materials and Methods

6.3.1. Seed material

The seeds of 115 Sardinian cultivars of *V. v. vinifera* were collected at the time of natural repining throughout the whole Sardinian region (Table 1 and Fig. 1).

The necessity to study and characterize vegetal material collected in different localities and different ecological conditions, induced the use of different sampling methods, following ethic-scientific criteria, anyway (Guarino et al. 1995; Bacchetta et al. 2006, 2008b).

Table 1 - Name code, grape variety name, colour (B = black; W = white), grape type (TG = table grape; WG = wine grape), wine type (TW = table wine; DW = moscat or dessert wine) and distribution of *V. v. vinifera* studied cultivars.

Name code	Grape variety	Grape colour	Consumption aptitude		Distribution
			Grape type	Wine type	
AGA	Aghina de Gerusalemme di Abbasanta	W	TG	-	Barigadu
AcA	Aghina'e cressia di Abbasanta	B	TG	-	Barigadu
AbT	Albacanna di Triei	W	TG	-	Ogliastra
Al	Alicante	W	WG	TW	Sardegna
AvB	Alvaranzeniadu bianco di Bosa	W	WG	DW	Planargia
AnL	Aniga di Lanusei	B	WG	TW	Mandrolisai
ApN	Apesorgia nera	B	TG	-	Campidano di Cagliari
ArB	Aregu biancu	W	WG	DW	Barbagia di Seùlo
Av	Arvesiniadu	W	WG	DW	Goceano
AxF	Axina de Francia	W	WG	TW	Campidano di Cagliari
Axt	Axina de tres bias	B	TG	-	Campidano di Cagliari
BrS	Barbera Sarda	B	WG	TW	Parteòlla
BnC	Bianca di Chilivri	W	TG/WG	TW	Baronie

BnL	Bianca di Lodine	W	WG	TW	Barbagia di Ollolai
BnP	Bianca di Padria	W	WG	TW	Meilogu
BnM	Bianca pelosa di Montresta	W	WG	TW	Planargia
CbS	Caddiu bianco di Serri	W	WG	TW	Sarcidano
CgN	Cagnulari di Nurri	B	WG	TW	Sarcidano
ClS	Calabresa di Seulo	W	WG	TW	Barbagia di Seùlo
CnB	Cannonatu anticu di Bitti	B	WG	TW	Barbagia di Nuoro
Cn	Cannonau	B	WG	TW	Barbagia (Barbagia di Belvì + Barbagia di Nuoro+Barbagia di Ollolai + Barbagia di Seùlo)
CnO	Cannonau bianco di Oliena	W	WG	TW	Barbagia (Barbagia di Belvì + Barbagia di Nuoro+Barbagia di Ollolai + Barbagia di Seùlo)
CnT	Cannonau bianco di Trieri	W	WG	TW	Ogliastra
CnS	Cannonau nero di Sestu	B	WG	TW	Campidano di Cagliari
CrT	Canulare di Triei	B	WG	TW	Ogliastra
Cs	Carenisca	B	WG	TW	Sulcis
CcM	Caricagiola di Monti	B	WG	TW	Monteacuto
Cd	Codronisca	W	WG	DW	Campidano di Cagliari
Cob	Corniola Bianca	W	TG	-	Parteòlla
CfO	Corofulu di Oliena	W	TG	-	Barbagia di Ollolai
CuU	Cuccuau di Ula	W	WG	TW	Barigadu
Cl	Culupuntu	W	WG	TW	Ogliastra
FlC	Falso Canulare di Triei	B	WG	TW	Ogliastra
FlG	Falso Gregu	B	WG	TW	Campidano di Cagliari
FdS	Fiudedda di Sini	B	WG	TW	Marmilla
GlE	Galoppu di Escalaplano	W	TG	-	Sarrabus-Gerrei
GlN	Galoppu di Nurri	W	TG	-	Sarcidano
Gr	Girò	B	WG	DW	Campidano di Cagliari
GrG	Girò di Gonnos	B	WG	DW	Campidano di Sanluri
Grm	Girò morbido di Serri	B	WG	TW	Sarcidano
Grs	Girò scuro di Serri	B	WG	DW	Sarcidano
GzS	Granatza Aregu di Seulo	W	WG	DW	Barbagia di Seùlo

GzM	Granatza di Mamoiada	W	WG	DW	Barbagia di Ollolai
GzG	Granazza di Garaumele	W	WG	DW	Barbagia di Ollolai
GB	Gregu bianco	W	WG	DW	Campidano di Cagliari e Oristano
GNC	Gregu Nieddu del Campidano	B	WG	TW	Campidano di Cagliari e Oristano
GNS	Gregu Nieddu di Serramanna	B	WG	TW	Campidano di Cagliari
LcA	Lacconarzu di Abbasanta	W	WG	TW	Barigadu
Lx	Licronaxiu	W	WG	TW	Campidano di Oristano
LxN	Licronaxiu nero Nuraxinieddu	B	WG	TW	Campidano di Oristano
LgA	Luglienca di Abbasanta	W	WG	TW	Barigadu
Mz	Mizu	W	WG	TW	Campidano Campidano (Campidano di Cagliari + Campidano di Oristano + Campidano di Sanluri) e Marmilla
MB	Monica Bianca	W	WG	TW	Campidano (Campidano di Cagliari + Campidano di Oristano + Campidano di Sanluri)
ME	Monica di Escalaplano	B	WG	TW	Sarrabus e Gerrei
MSl	Monica di Seulo	B	WG	TW	Barbagia di Seùlo
MSr	Monica di Sorgono	B	WG	TW	Mandrolisai
MN	Monica nera	B	WG	TW	Campidano di Cagliari
Mb	Moscatello bianco	W	WG	DW	Campidano (Campidano di Cagliari + Campidano di Oristano + Campidano di Sanluri)
MnG	Moscatello nero di Genuri	B	WG	DW	Marmilla
MrS	Moscatello rosso di Seulo	B	WG	DW	Barbagia di Seùlo
MbG	Moscato bianco di Genuri	W	WG	DW	Marmilla
MbS	Moscato bianco di Sini	W	WG	DW	Marmilla
MFn	Moscato di Fonni	W	WG	DW	Barbagia di Ollolai
MLd	Moscato di Lodine	W	WG	DW	Barbagia di Ollolai
MPt	Moscato di Pattada	W	WG	DW	Monteacuto
MT1	Moscato di Tempio 1	W	WG	DW	Gallura

MT2	Moscato di Tempio 2	W	WG	DW	Gallura
MnS	Moscato nero di Sini	B	WG	DW	Marmilla
MnU	Moscato nero di Ulatirso	B	WG	DW	Barigadu
MSP	Moscato su pinu di Mamoiada	W	WG	DW	Barbagia di Ollolai
MuS	Muristeddu di Sorgono	B	WG	TW	Mandrolisai
Mu	Muristellu	B	WG	TW	Mandrolisai
Ms	Mustiosa	B	WG	TW	Barbagia di Nuoro
Ns	Nasco	W	WG	DW	Campidano di Cagliari
NsA	Nasco nero di Abbasanta	B	WG	TW	Barigadu
NeA	Nera di Abbasanta	B	WG	TW	Barigadu
NeB	Nera di Bosa	B	WG	TW	Planargia
NeE	Nera di Escalaplano	B	WG	TW	Sarrabus-Gerrei
NeJ	Nera di Janna Ritha	B	WG	TW	Baronie
NeO	Nera di Orosei	B	WG	TW	Baronie
NeS	Nera di Sini	B	WG	TW	Marmilla
NeM	Nera glabra di Modolo	B	WG	TW	Planargia
NeL	Nera liscia di Montresta	B	WG	TW	Planargia
NeT	Nera tomentosa	B	WG	TW	Planargia
NSl	Niedda carta di Seulo	B	WG	TW	Barbagia di Seùlo
NNr	Nieddu mannu di Nurri	B	WG	TW	Sarcidano
NPt	Nieddu mannu di Pattada	B	WG	TW	Monteacuto
NPS	Nieddu Pedra Serra	B	WG	TW	Campidano di Sanluri
NPl	Nieddu Polchino	B	WG	TW	Monteacuto
Nr	Nuragus	W	WG	TW	Campidano di Cagliari
NrA	Nuragus Arrubiu	W	WG	TW	Campidano di Cagliari
Nrt	Nuragus Moscatello	W	WG	TW	Sarcidano
Nrd	Nuragus Muscadeddu	W	WG	TW	Sarrabus-Gerrei
NrS	Nuragus nero di Sini	B	WG	TW	Marmilla
NrR	Nuragus rosso rompizzolla	W	WG	TW	Campidano di Cagliari
ObO	Ocre e boe di Orosei	B	TG	-	Baronie
OlT	Olopo di Triei	W	TG	-	Ogliastra
PzU	Panzale di Ula	W	WG	TW	Barigadu

PsN	Pascale di Nurri	W	TG/WG	TW	Sarcidano
PrN	Primidivu Nieddu	B	TG/WG	TW	Meilogu
RmS	Remungiau di Serri	W	WG	DW	Sarcidano
RtM	Retagliaddu di Monti	W	WG	TW	Monteacuto
SpS	Salude e passa di Serramanna	B	WG	TW	Campidano di Cagliari
Sm	Semidano	W	WG	TW	Marmilla
Sn	Sinnidanu	W	WG	TW	Baronie
TrS	Teresina di Sini	B	WG	TW	Marmilla
TtG	Tittiacca di Gonnos	W	TG	-	Campidano di Sanluri
TtS	Tittiacca verde di Serramanna	W	TG	-	Campidano di Cagliari
TtM	Tittibacchina di Mamoiada	W	TG	-	Barbagia di Ollolai
VrV	Vernaccia bidri di Villasor	W	WG	DW	Campidano di Cagliari
VrE	Vernaccia di Escalaplano	W	WG	DW	Sarrabus-Gerrei
VrR	Vernaccia di S. Rosalia	W	WG	DW	Ogliastra
VrS	Vernaccia di Solarussa	W	WG	DW	Campidano di Oristano
VrO	Vernazza di Orosei	W	WG	DW	Baronie
Vrt	Vertudi	B	WG	TW	Sulcis

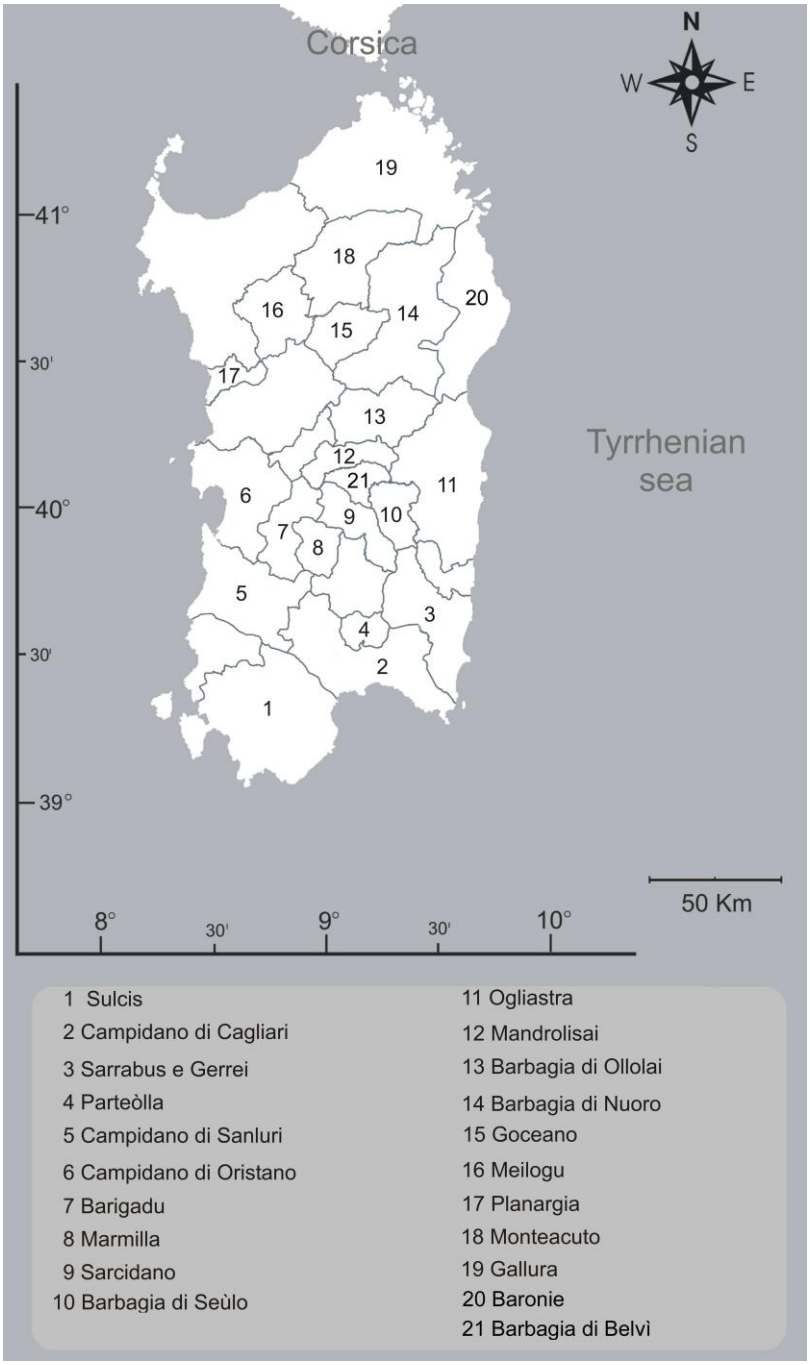

Figure 1. Sardinian cultivar distribution

6.3.2. Seed size, shape and colour analysis

Digital images of seed samples were acquired using a flatbed scanner (Epson GT-15000) with a digital resolution of 200 dpi and a scanning area not exceeding 1024×1024 pixel. Image acquisition was performed before drying the seeds at 15°C to 15% of R.H. to avoid spurious variation in dimension, shape and colour. Moreover, before image acquisition, the scanner was calibrated for colour matching following the protocol of Shahin and Symons (2003) as suggested by Venora et al. (2009).

Samples consisting of 100 seeds were captured and used for the digital image analysis. In order to represent the whole variability of each accession, the seed samples were acquired three times, randomly disposing them on the flatbed tray. A total of over 87,000 statistical cases were analysed.

Digital images of seeds were processed and analysed using the software package KS-400 V. 3.0 (Carl Zeiss, Vision, Oberkochen, Germany). A macro specifically developed for the characterization of wild seeds (Bacchetta et al. 2008a), and later modified to measure further twenty morpho-colorimetric seed features (Mattana et al. 2008), was adapted to perform automatically all the analysis procedures, reducing the execution time and contextually mistakes in the analysis process (Grillo et al. 2010).

Moreover, the binary images obtained by the segmentation process during the image analysis of the seeds, were redefined to 400 dpi to enhance the image definition and apply the Elliptic Fourier Descriptors (EFD) method, to increase the number of discriminant parameters (Tab. 2) (Bacchetta et al. 2009; 2010). As described by Orrù et al. (2012), this method allows to describe the boundary of the seed projection, as an array of complex numbers which correspond to the pixels position of the seed boundary. According to Terral et al. (2010) findings, about the use of number of harmonics for an optimal description of seed outlines, in order to minimize the measurement errors and optimizes the efficiency of shape reconstruction, 20 harmonics were used to define the seed boundaries, obtaining further 80 parameters useful to discriminate among the studied cultivars of *Vitis*. A total of 113 morpho-colorimetric features were measured for each seed.

Table 2. List of morpho-colorimetric features measured on seeds, excluding the 80 Elliptic Fourier Descriptors.

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

6.3.3. Statistical analysis

The obtained data were used to assemble a database of morpho-colorimetric and EFDs data . Using the SPSS software package release 15 (SPSS Inc. 1989-2006), the data were statistically elaborated applying the stepwise *Linear Discriminant Analysis* (LDA) method, to compare the investigated cultivars. This approach is commonly used to classify/identify unknown groups characterized by quantitative and qualitative variables (Fisher 1936, 1940). On the basis of all measured features, the stepwise method identifies and selects the most statistically significant of them to use for the seed sample identification. This method starts with a model that does not include any of the variables, adding step by step one more, until no remaining variables are able to increase the discrimination ability, stopping the process (Venora et al. 2009b).

Finally, a cross-validation procedure was applied to verify the performance of the identification system, testing individual unknown cases and classifying them on the basis of all others (SPSS 1999).

6.3.4. Grouping procedure

Due to the large number of data, the discriminatory steps were executed distinguishing between black and white berry cultivars, allowing an more easy identification of the hypotetical synonymy goups. Moreover, preliminar comparisons were executed distinguishing among consumption aptitude of the grapevine cultivars. Following historical notations it was possible differentiate between table grape and wine grape, and between table wine and moscat wine or dessert wine. In a final step, the so formed preliminar hypotetical groups were compared all together, only maintaining a distinction between white and black berry cultivars.

6.4. Results and Discussion

Using the same approach that many times have been applied to solve cases of homonymy and synonymy on the basis of molecular markers, morpho-colorimetric features were used to identify hypothetic grapevine cultivar synonymy groups. A total of 113 morpho-colorimetric features was used to characterize the germplasm of the 115 studied Sardinian grapevine cultivars (Tab. 1).

To make easier and flowing the comparing and grouping procedures among the analysed grapevine cultivars, they were preliminarly distinguished and slpitted in 54 black berry and 61 white berry cultivars.

Using historical and cultural information, a first statistical comparison was executed on the basis of the consumption aptitude, distinguishing among table grape, wine grape and double aptitude grape. Table 3 shows the percentages of correct identification of the three grape categories, making a distinction for the berry colour. The high overall percentages of correct identification both for black and for white berry cultivars are due to the quite perfect classification of wine grape seeds, that is the most large group. Instead, the low recognition performance for the two other partnership classes, probably ensues from the non univocal popular origin of the consumption aptitude information, collected in different areas of the Sardinian region. Anyway, this comparison is helpful to the correct assemblage of the hypotetical synonymy groups.

Table 3 - Statistical comparisons among the analysed grapevine cultivars, distinguished for the berry colour, on the basis of the consumption aptitude (TG = table grape; WG = wine grape; TG/WG = double aptitude). In parentheses, the number of seeds.

		WG	**TG**	**TG/WG**	**Total**
black berry cultivars	**WG**	**96.5% (33.725)**	2.6% (904)	0.9% (315)	100.0% (34.944)
	TG	64.5% (2.110)	**35.3% (1.154)**	0.2% (8)	100.0% (3.272)
	TG/WG	64.8% (386)	0.2% (1)	**35.1% (209)**	100.0% (596)
	Overall				**90.4% (38.812)**
		WG	**TG**	**TG/WG**	**Total**
white berry cultivars	**WG**	**97.6% (39407)**	2.3% (944)	0.1% (38)	100.0% (40389)
	TG	60.0% (3497)	**40.0% (2329)**	-	100.0% (5826)
	TG/WG	95.1% (855)	-	**4.9% (44)**	100.0% (899)
	Overall				**88.7% (47114)**

Comparing exclusively the table grapevine cultivars, distinguished for the berry colour (Tab. 4), it is possible to suppose that the four black berry cultivars: Apesorgia near (ApN), Axina de tres bias (Axt), Aghina'e cressia di Abbasanta (AcA) and Ocre e boe di Orosei (ObO) are independent on one another; while regarding the white berry cultivars, it seems that two hypotetical little groups are formed. Tittiacca di Gonnos (TtG) and Tittiacca verde di Serramanna (TtS) show good performance of correct identification (75.6% and 75.7%, respectively), but percentage of misattribution of over 21% one which other. Similarly, Corofulu di Oliena (CfO), Galoppu di Escalaplano (GlE), Galoppu di Nurri (GlN) and Tittibacchina di Mamoiada (TtM) reveal considerable misidentification percentage each other, included between 2.7% and 16.0% (Tab. 4).

Table 4. Statistical comparisons among the analysed table grapevine cultivars, distinguished for the berry colour. In parentheses, the number of seeds.

		ApN		**Axt**		**AcA**		**ObO**		**Total**	**Total**
black berry cultivars	**ApN**	**95.5% (1.138)**		4.0% (48)		0.5% (6)		-		100.0% (1.192)	100.0% (1.192)
	Axt	3.1% (37)		**92.1% (1.095)**		3.8% (45)		1.0% (12)		100.0% (1.189)	100.0% (1.189)
	AcA	2.2% (13)		7.1% (42)		**90.7% (536)**		-		100.0% (591)	100.0% (591)
	ObO	-		3.0% (9)		-		**97.0% (291)**		100.0% (300)	100.0% (300)
	Overall										
		Cob	**GlE**	**GlN**	**TtG**	**TtS**	**AGA**	**CfO**	**OlT**	**TtM**	**Total**
white berry cultivars	**Cob**	**90.5% (948)**	0.7% (7)	0.8% (8)	-	-	2.1% (22)	0.3% (3)	4.7% (49)	1.0% (11)	100.0% (1.048)
	GlE	0.3% (2)	**70.5% (422)**	16.0% (96)	-	-	1.5% (9)	2.7% (16)	0.2% (1)	8.8% (53)	100.0% (599)
	GlN	-	10.7% (67)	**72.4% (452)**	-	-	0.6% (4)	5.8% (36)	-	10.4% (65)	100.0% (624)
	TtG	1.8% (10)	-	-	**75.6% (428)**	21.7% (123)	0.4% (2)	-	0.5% (3)	-	100.0% (566)
	TtS	0.8% (5)	0.2% (1)	-	21.1% (125)	**75.7% (449)**	1.5% (9)	-	0.5% (3)	0.2% (1)	100.0% (593)
	AGA	0.7% (4)	1.5% (9)	1.0% (6)	-	-	**86.0% (516)**	2.0% (12)	7.5% (45)	1.3% (8)	100.0% (600)
	CfO	-	3.8% (23)	9.2% (55)	-	-	0.7% (4)	**76.1% (456)**	1.5% (9)	8.7% (52)	100.0% (599)
	OlT	8.5% (51)	0.5% (3)	1.5% (9)	-	-	7.0% (42)	2.2% (13)	**79.9% (477)**	0.3% (2)	100.0% (597)
	TtM	-	12.2% (73)	9.0% (54)	-	-	0.7% (4)	8.8% (53)	0.7% (4)	**68.7% (412)**	100.0% (600)
	Overall										**78.3% (5.826)**

The 100.0% of correct identification achieved from the comparison between the two double aptitude white grapevine cultivars (Tab. 5) proves that Pascale di Nurri (PsN) and Bianca di Chilivri (BnC) have not any degree of morphological similarity. No comparison among double aptitude black berry cultivars was possible, due to the availability of only one cultivar benging to this category.

Table 5 - Statistical comparisons among the analysed double aptitude white grapevine cultivars. In parentheses, the number of seeds.

	PsN	**BnC**	**Total**
PsN	**100.0% (599)**	**-**	100.0% (599)
BnC	**-**	**100.0% (300)**	100.0% (300)
Overall	**-**	**-**	**100.0% (899)**

A statistical comparison among the wine black grapevine cultivars was implemented in order to recognize possible synonymy groups (Tab. 6). The evaluation of the performance of each cultivar and above all the assessment of the relative mistakes allowed to hypotize some assemblages. Falso Gregu (FlG), Nasco nero di Abbasanta (NsA), Nera tomentosa (NeT), Nieddu mannu di Nurri (NNr), Nieddu mannu di Pattada (NPt) and Nieddu Pedra Serra (NPS) could be grouped in an unique synonymy cluster, confirming and enriching the achievements of De Mattia et al. (2007) and Orrù et al. (2012b). Similar evaluations allowed to hypotize a few of other possible synonymy groups. Aniga di Lanusei (AnL), Cannonau (Cn), Cannonau nero di Sestu (CnS) and Nera liscia di Montresta (NeL) could be considered as belonging to the same synonymy group, as well as Cagnulari di Nurri (CgN), Canulare di Triei (CrT) and Falso Canulare di Triei (FlC). Also the cultivars Carenisca (Cs), Muristellu (Mu) and Muristeddu di Sorgono (MuS) seems to belong to the same group, just as Gregu Nieddu del Campidano (GNC), Gregu Nieddu di Serramanna (GNS) and Vertudi (Vrt), which misattributions are crossed. The evaluation of the results of this preliminary comparison allowed to suppose many other synonymy groups: according to De Mattia et al. (2007) and Orrù et al. (2012b), Nera di Bosa (NeB) and Nieddu Polchinu (NPl) seem to be morphologically similar also to Cannonatu anticu di Bitti (CnB), as well as Monica di Escalaplano (ME) and Monica di Seulo (MSl), enriched with the cultivar Monica nera (MN). Other little similarity clusters could be constituted by Fiudedda di Sini (FdS) and Teresina di

Sini (TrS); Monica di Sorgono (MSr) and Nera di Abbasanta (NeA); Barbera Sarda (BrS) and Nera di Escalaplano (NeE); Nera di Sini (NeS) and Salude e passa di Serramanna (SpS); and finally Nera glabra di Modolo (NeM), Niedda carta di Seulo (NSl) and Nuragus nero di Sini (NrS) (Tab. 6).

Table 6. Statistical comparisons among the analysed black grapevine cultivars. Percentage and number of seeds in parenthesis.

	BrS	CgN	Cn	Cs	CcM	FlG	GNC	MN	Mu	NeB	Vrt	AnL	CnS	CrT	FlC	FdS	Grm	GNS	LxN	ME	MSl	MSr	MuS	NsA	NeA	NeE	NeS	NeM	NeL	NeT	NSl	NPS	NNr	NPt	NPl	NrS	SpS	TrS	CnB	Ms	NeO	NeJ	Total
BrS	**69.8 (733)**	-	0.3 (5)	0.5 (5)	0.1 (1)	0.6 (6)	1.9 (20)	3.0 (31)	0.4 (4)	3.0 (32)	1.4 (15)	-	-	-	-	-	1.7 (18)	0.3 (3)	-	0.5 (5)	3.0 (32)	0.2 (2)	-	0.8 (8)	1.8 (19)	3.0 (31)	0.9 (9)	1.5 (16)	0.3 (3)	-	-	-	0.8 (8)	-	1.1 (12)	-	3.0 (32)	0.1 (1)	0.1 (1)	-	-	-	100.0 (1.050)
CgN	-	**80.4 (481)**	-	0.2 (1)	1.8 (11)	-	0.3 (2)	-	-	1.0 (6)	0.8 (5)	-	-	3.2 (19)	-	2.0 (12)	1.2 (7)	0.8 (5)	0.7 (4)	-	-	0.7 (4)	1.0 (6)	-	0.8 (5)	0.7 (4)	-	2.5 (15)	-	-	0.2 (1)	-	0.8 (5)	-	-	0.3 (2)	-	0.3 (2)	-	-	0.2 (1)	-	100.0 (598)
Cn	0.2 (4)	-	**62.8 (1.116)**	-	0.8 (14)	0.6 (10)	3.6 (64)	-	-	-	2.6 (46)	7.0 (124)	4.7 (84)	-	-	-	0.1 (2)	1.4 (24)	0.6 (10)	0.1 (2)	0.1 (2)	-	0.7 (12)	-	4.8 (86)	0.1 (2)	-	2.8 (50)	5.9 (104)	-	0.2 (4)	-	0.1 (2)	-	-	0.6 (10)	-	-	0.1 (2)	-	-	0.1 (2)	100.0 (1.776)
Cs	0.5 (5)	-	-	**24.7 (256)**	0.2 (2)	1.9 (20)	0.2 (2)	-	26.2 (272)	2.2 (23)	2.5 (26)	-	-	0.3 (3)	-	3.9 (41)	0.1 (1)	2.1 (22)	0.3 (3)	0.1 (1)	0.5 (5)	0.8 (8)	19.2 (199)	1.3 (13)	2.3 (24)	3.2 (33)	-	0.7 (7)	0.2 (2)	0.8 (8)	0.1 (1)	0.5 (5)	0.6 (6)	0.5 (5)	1.0 (10)	0.7 (7)	-	1.7 (18)	0.5 (5)	-	0.5 (5)	-	100.0 (1.038)
CcM	0.2 (1)	0.5 (3)	0.8 (5)	-	**63.4 (377)**	-	1.5 (9)	-	-	0.3 (2)	1.3 (8)	2.7 (16)	0.8 (5)	2.5 (15)	0.7 (4)	5.9 (35)	0.2 (1)	3.0 (18)	2.2 (13)	-	-	0.7 (4)	2.5 (15)	-	0.3 (2)	0.2 (1)	0.2 (1)	2.5 (15)	0.2 (1)	1.5 (9)	0.7 (4)	0.2 (1)	1.0 (6)	2.4 (14)	-	1.0 (6)	-	0.5 (3)	-	-	0.2 (1)	-	100.0 (595)
FlG	0.8 (7)	0.1 (1)	0.1 (1)	2.1 (19)	0.7 (6)	**48.9 (437)**	0.4 (4)	0.8 (7)	0.8 (7)	1.2 (11)	4.6 (41)	-	-	0.2 (2)	0.2 (2)	1.0 (9)	0.1 (1)	0.6 (5)	0.8 (7)	-	0.8 (7)	1.1 (10)	0.1 (1)	9.4 (84)	0.8 (7)	1.1 (10)	0.4 (4)	-	0.6 (5)	3.6 (32)	0.1 (1)	8.2 (73)	2.1 (19)	4.9 (44)	2.1 (19)	0.2 (2)	0.2 (2)	0.2 (2)	0.1 (1)	-	0.4 (4)	-	100.0 (894)
GNC	2.3 (27)	-	2.1 (25)	1.7 (20)	1.2 (14)	5.4 (65)	**45.6 (547)**	-	2.1 (25)	0.3 (4)	13.3 (160)	1.9 (23)	0.3 (4)	-	-	0.3 (4)	0.2 (2)	9.4 (113)	0.1 (1)	-	0.2 (2)	0.9 (11)	1.1 (13)	0.3 (3)	4.3 (51)	0.7 (8)	-	2.9 (35)	0.2 (2)	0.3 (4)	0.1 (1)	0.6 (7)	0.3 (3)	0.6 (7)	0.3 (3)	0.3 (3)	0.8 (10)	0.2 (2)	-	-	-	-	100.0 (1.199)
MN	4.3 (26)	-	0.2 (1)	0.8 (5)	0.2 (1)	0.3 (2)	0.2 (1)	**38.0 (228)**	-	0.2 (1)	0.7 (4)	-	-	-	-	-	0.3 (2)	0.2 (1)	0.2 (1)	20.3 (122)	12.7 (76)	0.7 (4)	0.5 (3)	0.8 (5)	1.2 (7)	2.2 (13)	2.2 (13)	1.3 (8)	-	0.2 (1)	0.7 (4)	0.2 (1)	3.8 (23)	0.2 (1)	3.2 (19)	0.2 (1)	3.2 (19)	-	-	0.8 (5)	0.2 (1)	0.2 (1)	100.0 (600)
Mu	0.7 (5)	-	-	15.1 (113)	-	1.7 (13)	-	-	**72.1 (539)**	-	0.8 (6)	-	-	0.1 (1)	-	1.6 (12)	-	-	-	-	0.1 (1)	-	5.2 (39)	-	0.5 (4)	-	-	0.3 (2)	0.1 (1)	-	0.1 (1)	0.5 (4)	-	-	-	0.7 (5)	-	0.3 (2)	-	-	-	-	100.0 (748)
NeB	2.0 (12)	0.2 (1)	0.2 (1)	0.3 (2)	0.5 (3)	0.8 (5)	-	4.5 (27)	-	**43.0 (258)**	-	-	-	0.2 (1)	-	1.3 (8)	7.3 (44)	0.3 (2)	1.0 (6)	2.7 (16)	0.5 (3)	-	2.7 (16)	-	0.3 (2)	5.8 (35)	3.8 (23)	0.2 (1)	-	1.7 (10)	1.2 (7)	0.8 (5)	1.7 (10)	-	14.2 (85)	-	1.8 (11)	0.5 (3)	0.5 (3)	-	-	-	100.0 (600)
Vrt	2.4 (29)	0.3 (3)	2.8 (34)	2.9 (35)	1.8 (22)	1.1 (13)	12.3 (148)	-	0.8 (10)	0.4 (5)	**45.5 (546)**	0.1 (1)	0.3 (4)	-	-	1.1 (13)	0.3 (4)	12.4 (149)	0.6 (7)	-	0.2 (2)	0.2 (2)	4.8 (58)	0.2 (2)	4.6 (55)	0.5 (6)	0.2 (2)	0.8 (10)	0.1 (1)	0.5 (6)	0.2 (2)	0.2 (2)	1.6 (19)	0.6 (7)	-	0.2 (2)	-	-	-	-	-	0.1 (1)	100.0 (1.200)
AnL	-	-	19.0 (114)	-	0.7 (4)	-	1.2 (7)	-	-	-	0.3 (2)	**48.2 (289)**	16.0 (96)	-	-	-	-	2.2 (13)	1.0 (6)	-	-	-	-	-	3.0 (18)	-	-	0.3 (2)	7.3 (44)	0.3 (2)	0.2 (1)	-	0.2 (1)	-	-	-	-	-	0.2 (1)	-	-	-	-	100.0 (600)
CnS	-	-	10.9 (65)	-	0.2 (1)	0.7 (4)	0.7 (4)	-	-	-	0.2 (1)	26.7 (160)	**34.1 (204)**	-	-	0.2 (1)	-	2.0 (12)	0.5 (3)	-	-	-	0.2 (1)	-	4.7 (28)	-	-	-	17.5 (105)	0.5 (3)	0.5 (3)	-	0.5 (3)	-	-	-	-	0.2 (2)	-	-	-	-	-	100.0 (599)
CrT	-	6.8 (41)	-	-	0.3 (2)	-	-	-	-	0.3 (2)	-	-	-	**63.2 (380)**	13.5 (81)	5.7 (34)	-	0.2 (1)	1.2 (7)	-	-	-	-	-	-	-	-	0.8 (5)	-	-	1.2 (7)	-	0.2 (1)	0.2 (1)	-	1.7 (10)	-	2.8 (17)	-	-	-	2.0 (12)	100.0 (601)
FlC	-	0.7 (2)	-	-	-	-	-	-	-	-	-	-	-	29.0 (87)	**61.0 (183)**	6.3 (19)	-	-	0.3 (1)	-	-	-	-	-	-	-	-	0.3 (1)	-	-	1.0 (3)	-	-	-	-	0.3 (1)	-	0.7 (2)	-	-	0.3 (1)	-	100.0 (300)
FdS	-	0.3 (4)	-	1.8 (22)	2.3 (28)	0.2 (2)	-	-	-	0.2 (2)	0.3 (4)	-	-	4.3 (52)	0.7 (8)	**72.9 (872)**	-	0.8 (10)	0.5 (6)	-	0.3 (4)	0.3 (4)	5.4 (64)	-	0.3 (4)	0.2 (2)	-	0.2 (2)	0.2 (2)	1.3 (16)	0.3 (4)	0.5 (6)	0.5 (6)	0.8 (10)	-	0.5 (6)	-	4.7 (56)	-	-	-	-	100.0 (1.196)
Grm	2.3 (14)	1.3 (8)	0.5 (3)	0.5 (3)	-	-	-	0.7 (4)	-	6.0 (36)	0.3 (2)	-	-	-	-	-	**71.2 (428)**	1.0 (6)	2.7 (16)	-	0.7 (4)	-	1.8 (11)	-	0.2 (1)	0.8 (5)	1.7 (10)	2.7 (16)	0.5 (3)	-	0.3 (2)	-	0.7 (4)	-	0.8 (5)	0.2 (1)	3.2 (19)	-	-	-	-	100.0 (601)
GNS	0.7 (4)	-	1.5 (9)	1.8 (11)	6.9 (41)	0.7 (4)	11.7 (70)	-	-	2.0 (12)	16.7 (100)	0.8 (5)	0.8 (5)	0.2 (1)	-	1.8 (11)	0.8 (5)	**30.9 (185)**	-	-	0.2 (1)	1.0 (6)	6.9 (41)	0.7 (4)	6.4 (38)	0.3 (2)	0.2 (1)	1.5 (9)	1.2 (7)	-	0.3 (2)	0.3 (2)	1.0 (6)	0.2 (1)	1.7 (10)	-	-	0.3 (2)	0.3 (2)	-	0.2 (1)	-	100.0 (598)
LxN	-	1.5 (9)	0.8 (5)	0.2 (1)	6.2 (37)	0.2 (1)	0.8 (5)	-	-	0.2 (1)	0.5 (3)	1.3 (8)	0.2 (1)	0.5 (3)	0.8 (5)	4.2 (25)	3.2 (19)	2.0 (12)	**57.8 (347)**	-	-	0.3 (2)	3.8 (23)	0.2 (1)	0.3 (2)	0.3 (2)	1.5 (9)	0.2 (1)	0.7 (4)	0.5 (3)	7.5 (45)	0.5 (3)	0.7 (4)	0.5 (3)	0.3 (2)	2.0 (12)	-	0.2 (1)	-	-	0.2 (1)	-	100.0 (600)
ME	0.5 (3)	0.2 (1)	-	0.2 (1)	-	-	-	9.0 (54)	-	0.7 (4)	0.3 (2)	-	-	0.2 (1)	0.2 (1)	-	-	-	-	**67.5 (405)**	2.7 (16)	0.2 (1)	-	1.0 (6)	1.5 (9)	0.8 (5)	0.2 (1)	2.8 (17)	-	0.2 (1)	0.3 (2)	-	5.2 (31)	1.0 (6)	-	-	1.3 (8)	-	-	-	-	3.8 (23)	0.3 (2)	100.0 (600)
MSl	8.7 (52)	0.2 (1)	-	1.2 (7)	-	7.6 (45)	0.5 (3)	13.8 (82)	-	0.2 (1)	1.0 (6)	-	-	-	-	0.2 (1)	0.5 (3)	0.3 (2)	-	1.5 (9)	**28.9 (172)**	0.2 (1)	-	1.5 (9)	1.2 (7)	2.0 (12)	1.8 (11)	6.0 (36)	-	-	0.7 (4)	1.0 (6)	4.2 (25)	0.2 (1)	6.7 (40)	0.2 (1)	8.2 (49)	-	1.0 (6)	0.7 (4)	-	-	100.0 (596)
MSr	0.7 (4)	0.5 (3)	1.0 (6)	1.8 (11)	0.3 (2)	-	0.2 (1)	1.0 (6)	-	0.2 (1)	1.7 (10)	-	-	0.2 (1)	-	4.5 (27)	-	1.8 (11)	-	0.8 (5)	-	0.3 (2)	**53.8 (323)**	3.7 (22)	0.5 (3)	18.5 (111)	1.0 (6)	0.2 (1)	3.3 (20)	-	0.2 (1)	0.2 (1)	0.5 (3)	1.3 (8)	-	0.3 (2)	0.3 (2)	0.7 (4)	-	-	0.5 (3)	0.3 (2)	100.0 (600)
MuS	1.9 (22)	0.3 (4)	0.2 (2)	5.2 (62)	0.5 (6)	0.2 (2)	-	-	0.2 (2)	0.2 (2)	0.3 (4)	-	-	0.7 (8)	-	9.1 (108)	0.5 (6)	1.0 (12)	0.2 (2)	-	-	1.3 (16)	**67.3 (800)**	0.5 (6)	3.5 (42)	1.2 (14)	-	3.0 (36)	-	0.2 (2)	0.2 (2)	0.2 (2)	0.3 (4)	0.3 (4)	-	0.2 (2)	-	1.0 (12)	0.3 (4)	-	-	100.0 (1.188)
NsA	2.3 (14)	-	0.2 (1)	4.2 (26)	0.2 (1)	5.4 (33)	0.8 (5)	1.5 (9)	-	0.7 (4)	0.5 (3)	-	-	-	-	0.5 (3)	0.5 (3)	2.1 (13)	-	0.2 (1)	2.0 (12)	0.5 (3)	2.1 (13)	**38.1 (234)**	6.4 (39)	2.1 (13)	0.8 (5)	0.2 (1)	1.1 (7)	7.0 (43)	-	8.5 (52)	2.6 (16)	2.4 (15)	3.1 (19)	0.2 (1)	0.8 (5)	0.2 (1)	1.0 (6)	2.1 (13)	-	-	100.0 (614)
NeA	1.0 (12)	0.3 (4)	0.5 (6)	1.0 (12)	0.3 (4)	-	1.0 (12)	0.3 (4)	0.3 (4)	0.5 (6)	1.8 (22)	-	-	-	-	1.0 (12)	0.2 (2)	2.8 (34)	-	0.8 (10)	0.5 (6)	5.9 (70)	3.5 (42)	1.5 (18)	**64.4 (770)**	2.7 (32)	-	4.5 (54)	1.5 (18)	0.3 (4)	0.3 (4)	0.3 (4)	0.3 (4)	0.3 (4)	0.2 (2)	0.3 (4)	-	0.3 (4)	0.7 (8)	0.2 (2)	-	0.2 (2)	100.0 (1.196)
NeE	2.8 (17)	-	0.2 (1)	1.2 (7)	-	0.2 (1)	-	0.7 (4)	-	3.5 (21)	0.2 (1)	-	-	-	-	0.2 (1)	0.2 (1)	-	-	-	0.5 (3)	0.2 (1)	3.2 (19)	0.8 (5)	1.5 (9)	**77.5 (465)**	-	1.2 (7)	-	0.5 (3)	-	0.5 (3)	1.8 (11)	0.3 (2)	1.7 (10)	0.7 (4)	0.5 (3)	-	0.2 (1)	-	-	-	-	100.0 (600)
NeS	1.4 (8)	0.3 (2)	-	1.9 (11)	-	0.3 (2)	-	4.3 (25)	-	4.5 (26)	0.3 (2)	-	-	-	-	0.3 (2)	5.8 (34)	0.2 (1)	0.5 (3)	1.0 (6)	3.3 (19)	0.5 (3)	0.2 (1)	1.5 (9)	1.5 (9)	1.0 (6)	**44.7 (261)**	0.9 (5)	-	0.2 (1)	-	0.3 (2)	1.7 (10)	0.3 (2)	0.7 (4)	-	22.1 (129)	-	0.2 (1)	-	-	-	-	100.0 (584)
NeM	2.0 (12)	2.0 (12)	0.3 (2)	0.7 (4)	2.8 (17)	-	-	1.3 (8)	-	0.5 (3)	1.2 (7)	-	-	-	0.7 (4)	0.8 (5)	0.7 (4)	1.2 (7)	0.2 (1)	1.2 (7)	3.7 (22)	1.5 (9)	5.0 (30)	-	3.5 (21)	1.8 (11)	0.2 (1)	**58.5 (352)**	-	0.2 (1)	2.8 (17)	-	4.8 (29)	0.7 (4)	-	2.0 (12)	-	-	-	-	-	-	100.0 (602)
NeL	0.7 (4)	0.2 (1)	7.9 (47)	0.5 (3)	0.2 (1)	2.9 (17)	0.8 (5)	-	-	0.2 (1)	0.3 (2)	5.9 (35)	17.9 (106)	-	-	-	0.8 (5)	1.3 (8)	1.0 (6)	-	0.2 (1)	-	0.8 (5)	0.3 (2)	5.7 (34)	-	-	-	**48.6 (288)**	0.7 (4)	0.3 (2)	1.3 (8)	0.2 (1)	0.2 (1)	0.7 (4)	-	-	-	0.3 (2)	-	-	-	100.0 (593)
NeT	0.2 (1)	0.7 (4)	0.5 (3)	1.5 (9)	4.0 (24)	6.2 (37)	0.3 (2)	-	0.2 (1)	0.3 (2)	0.5 (3)	0.2 (1)	0.3 (2)	0.3 (2)	0.2 (1)	5.2 (31)	0.2 (1)	3.2 (19)	0.8 (5)	-	0.7 (4)	0.8 (5)	2.5 (15)	0.8 (5)	0.8 (5)	0.7 (4)	0.3 (2)	-	0.7 (4)	**27.9 (167)**	0.3 (2)	12.5 (75)	4.0 (24)	12.9 (77)	0.2 (1)	0.2 (1)	0.3 (2)	0.5 (3)	1.5 (9)	0.2 (1)	-	-	100.0 (599)
NSl	0.2 (1)	1.2 (7)	0.8 (5)	0.8 (5)	0.8 (5)	0.2 (1)	-	0.3 (2)	-	1.5 (9)	0.5 (3)	-	0.3 (2)	2.3 (14)	2.3 (14)	2.0 (12)	0.8 (5)	1.2 (7)	6.4 (38)	-	0.3 (2)	0.3 (2)	2.5 (15)	-	1.8 (11)	0.5 (3)	-	5.0 (30)	0.2 (1)	-	**53.9 (322)**	-	9.7 (58)	0.3 (2)	0.2 (1)	2.3 (14)	-	0.2 (1)	-	-	0.8 (5)	-	100.0 (597)
NPS	-	0.2 (1)	0.2 (1)	1.8 (11)	1.0 (6)	11.2 (67)	0.2 (1)	-	-	0.2 (1)	0.2 (1)	-	-	0.3 (2)	0.2 (1)	1.8 (11)	0.2 (1)	1.0 (6)	1.5 (9)	-	0.7 (4)	0.7 (4)	0.2 (1)	10.9 (65)	0.2 (1)	2.5 (15)	1.0 (6)	-	0.5 (3)	10.9 (65)	1.0 (6)	**39.2 (235)**	2.0 (12)	5.7 (34)	1.0 (6)	-	0.2 (1)	0.5 (3)	2.8 (17)	0.2 (1)	0.2 (1)	-	-	100.0 (599)
NNr	1.9 (11)	2.7 (16)	0.7 (4)	2.0 (12)	3.4 (20)	1.0 (6)	0.3 (2)	-	-	0.3 (2)	1.2 (7)	-	0.2 (1)	0.3 (2)	0.2 (1)	0.7 (4)	0.3 (2)	1.2 (7)	2.9 (17)	0.5 (3)	1.5 (9)	0.8 (5)	1.0 (6)	0.8 (5)	0.8 (5)	0.7 (4)	0.8 (5)	9.1 (54)	1.7 (10)	5.1 (30)	1.0 (6)	**51.9 (308)**	1.7 (10)	0.2 (1)	0.5 (3)	1.5 (9)	-	-	-	-	1.2 (7)	-	100.0 (593)
NPt	-	-	0.2 (1)	0.7 (4)	5.0 (30)	5.0 (30)	0.2 (1)	0.3 (2)	-	-	2.2 (13)	0.2 (1)	0.2 (1)	0.5 (3)	0.2 (1)	6.2 (37)	-	2.0 (12)	1.3 (8)	-	-	1.8 (11)	1.7 (10)	3.5 (21)	-	0.5 (3)	-	-	1.2 (7)	11.6 (69)	1.0 (6)	3.7 (22)	3.2 (19)	**43.5 (259)**	-	0.2 (1)	-	0.3 (2)	0.2 (1)	2.7 (16)	0.2 (1)	0.5 (3)	-	100.0 (595)
NPl	1.3 (8)	-	0.2 (1)	0.3 (2)	0.5 (3)	1.0 (6)	-	7.2 (43)	-	11.1 (66)	-	-	-	-	-	0.2 (1)	7.4 (44)	0.8 (5)	0.7 (4)	0.5 (3)	1.2 (7)	-	0.2 (1)	2.3 (14)	0.3 (2)	0.8 (5)	3.0 (18)	-	-	0.5 (3)	0.7 (4)	3.2 (19)	1.0 (6)	0.2 (1)	**49.5 (295)**	-	1.7 (10)	0.2 (1)	3.4 (20)	0.7 (4)	-	-	100.0 (596)
NrS	0.3 (2)	1.5 (9)	0.5 (3)	1.3 (8)	7.4 (44)	0.2 (1)	0.3 (2)	1.3 (8)	-	0.7 (4)	1.7 (10)	0.5 (3)	-	1.2 (7)	0.2 (1)	3.0 (18)	0.3 (2)	0.2 (1)	5.9 (35)	3.9 (23)	-	5.4 (32)	1.5 (9)	0.2 (1)	0.7 (4)	1.2 (7)	0.7 (4)	2.9 (17)	-	-	3.0 (18)	-	0.5 (3)	0.5 (3)	-	**48.2 (287)**	-	0.7 (4)	-	-	3.2 (19)	1.2 (7)	100.0 (596)
SpS	2.4 (14)	0.2 (1)	-	2.0 (12)	-	0.2 (1)	1.0 (6)	4.0 (24)	-	2.9 (17)	0.3 (2)	-	-	0.5 (3)	-	0.2 (1)	4.0 (24)	0.3 (2)	0.7 (4)	0.5 (3)	2.5 (15)	0.3 (2)	0.3 (2)	1.0 (6)	0.5 (3)	1.7 (10)	15.9 (94)	0.2 (1)	-	0.5 (3)	0.2 (1)	-	3.9 (23)	-	0.2 (1)	-	**53.5 (317)**	-	-	-	0.2 (1)	-	100.0 (593)
TrS	-	0.8 (5)	-	1.0 (6)	2.7 (16)	0.7 (4)	-	0.2 (1)	-	0.5 (3)	0.3 (2)	-	-	2.5 (15)	0.8 (5)	31.9 (190)	-	0.2 (1)	1.5 (9)	-	-	2.0 (12)	2.5 (15)	0.5 (3)	0.5 (3)	-	-	-	-	0.3 (2)	0.2 (1)	0.8 (5)	-	2.5 (15)	0.2 (1)	0.3 (2)	-	**45.2 (269)**	0.2 (1)	0.5 (3)	0.3 (2)	0.2 (1)	100.0 (595)
CnB	-	-	-	0.3 (1)	-	-	-	-	-	6.7 (20)	-	-	-	-	-	0.3 (1)	-	1.0 (3)	-	-	-	-	0.3 (1)	2.7 (8)	1.3 (4)	-	-	-	-	2.7 (8)	-	8.0 (24)	-	-	0.2 (1)	-	-	**66.7 (200)**	1.0 (3)	-	-	-	100.0 (300)
Ms	-	-	-	-	-	1.3 (4)	-	0.3 (1)	-	-	-	-	-	0.3 (1)	0.7 (2)	0.7 (2)	-	-	-	1.0 (3)	-	1.0 (3)	0.7 (2)	0.3 (1)	2.7 (8)	-	-	0.3 (1)	0.3 (1)	-	1.0 (3)	-	3.3 (10)	-	-	-	0.3 (1)	1.7 (5)	**83.3 (250)**	0.7 (2)	-	100.0 (300)
NeO	-	0.9 (2)	-	-	0.5 (1)	-	-	0.5 (1)	-	0.5 (1)	0.5 (1)	-	-	1.4 (3)	-	1.9 (4)	-	0.5 (2)	0.5 (1)	1.4 (3)	-	1.4 (3)	-	0.5 (1)	0.5 (1)	-	0.5 (1)	0.5 (1)	-	2.8 (6)	-	3.7 (8)	2.3 (5)	-	-	-	0.5 (1)	-	**77.8 (168)**	0.5 (1)	100.0 (216)
NeJ	-	1.0 (2)	-	-	-	-	-	-	-	-	-	-	-	10.0 (21)	0.5 (1)	-	0.5 (1)	-	1.4 (3)	-	-	-	1.0 (2)	-	-	-	-	1.9 (4)	-	-	-	2.4 (5)	-	1.4 (3)	-	-	0.5 (1)	**79.5 (167)**	100.0 (210)
Overall																																										**54.3 (28.955)**

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Table 7. Statistical comparisons among the analysed white wine grape cultivars. Percentage and number of seeds in parenthesis.

	AxF	BnM	CnT	Cl	Lx	Mz	Nr	MB	Sm	Sn	AbT	Al	BnL	BnP	CbS	ClS	CuU	LcA	LgA	NrA	Nrt	Nrd	NrR	PzU	RtM	CnO	Total
AxF	**69.4 (832)**	0.6 (7)	0.8 (10)	0.1 (1)	0.7 (8)	4.1 (49)	0.4 (5)	4.6 (55)	2.6 (31)	1.5 (18)	0.7 (8)	5.3 (63)	0.4 (5)	-	0.6 (7)	0.3 (4)	3.3 (39)	0.5 (6)	0.2 (2)	0.4 (5)	0.3 (3)	0.5 (6)	0.2 (2)	2.3 (28)	0.4 (5)	-	100.0 (1.199)
BnM	0.5 (3)	**60.1 (358)**	1.2 (7)	4.9 (29)	14.1 (84)	0.5 (3)	0.3 (2)	0.8 (5)	-	0.5 (3)	0.5 (3)	0.2 (1)	1.2 (7)	-	7.0 (42)	0.7 (4)	2.9 (17)	-	-	-	0.5 (3)	1.7 (10)	0.8 (5)	0.8 (5)	0.8 (5)	-	100.0 (596)
CnT	-	0.2 (2)	**70.2 (842)**	0.3 (4)	0.2 (2)	0.2 (2)	0.3 (4)	0.5 (6)	0.3 (4)	8.7 (104)	0.5 (6)	2.8 (34)	0.2 (2)	-	0.7 (8)	0.8 (10)	-	0.5 (6)	-	0.2 (2)	-	-	-	0.7 (8)	5.0 (60)	7.8 (94)	100.0 (1.200)
Cl	-	4,5 (27)	2.2 (13)	**74.9 (450)**	7.3 (44)	-	0.3 (2)	-	-	0.2 (1)	0.2 (1)	-	0.5 (3)	-	1.5 (9)	-	-	-	-	2.2 (13)	-	1.2 (7)	1.0 (6)	-	2.2 (13)	2.0 (12)	100.0 (601)
Lx	1.7 (18)	12.3 (129)	0.3 (3)	5.7 (60)	**59.1 (618)**	0.5 (5)	3.8 (40)	1.0 (10)	0.3 (3)	1.8 (19)	0.3 (3)	0.3 (3)	0.8 (8)	-	2.7 (28)	0.2 (2)	0.4 (4)	-	0.2 (2)	0.9 (9)	1.6 (17)	3.2 (33)	2.4 (25)	0.1 (1)	0.5 (5)	-	100.0 (1.045)
Mz	3.8 (80)	0.2 (4)	0.1 (2)	-	0.6 (12)	**58.7 (1.232)**	0.9 (18)	7.3 (154)	11.6 (244)	1.0 (22)	1.1 (24)	1.0 (22)	0.6 (12)	-	2.0 (42)	0.1 (2)	2.3 (48)	0.9 (18)	2.5 (52)	0.4 (8)	1.0 (22)	2.2 (46)	1.0 (22)	0.4 (8)	0.2 (4)	-	100.0 (2.098)
Nr	2.0 (18)	0.5 (4)	1.1 (10)	0.9 (8)	6.6 (59)	5.5 (49)	**49.4 (439)**	1.8 (16)	2.1 (19)	4.8 (43)	0.5 (4)	0.5 (4)	1.7 (15)	-	1.0 (9)	0.1 (1)	0.3 (3)	0.1 (1)	0.6 (5)	3.3 (29)	7.7 (68)	5.1 (45)	2.6 (23)	0.1 (1)	1.0 (9)	0.7 (6)	100.0 (888)
MB	3.0 (37)	0.4 (5)	-	0.1 (1)	0.2 (2)	7.4 (91)	1.2 (15)	**67.1 (824)**	4.8 (59)	2.0 (24)	0.9 (11)	0.2 (3)	1.5 (19)	-	0.7 (8)	1.0 (12)	5.4 (66)	0.4 (5)	1.2 (15)	0.1 (1)	-	0.2 (3)	0.2 (2)	2.0 (24)	0.1 (1)	-	100.0 (1.228)
Sm	4.9 (51)	0.1 (1)	0.9 (9)	0.1 (1)	0.8 (8)	30.6 (321)	2.5 (26)	8.3 (87)	**38.2 (401)**	2.2 (23)	0.8 (8)	2.8 (29)	0.8 (8)	-	0.2 (2)	-	0.9 (9)	0.4 (4)	-	0.6 (6)	2.3 (24)	0.2 (2)	0.2 (2)	1.1 (12)	1.4 (15)	-	100.0 (1.049)
Sn	1.5 (18)	-	9.4 (112)	0.3 (3)	0.3 (4)	0.7 (8)	1.3 (15)	0.8 (10)	2.4 (29)	**66.2 (790)**	0.3 (4)	0.5 (6)	-	-	0.1 (1)	0.7 (8)	-	-	-	0.1 (1)	0.2 (2)	-	-	5.3 (63)	0.6 (7)	9.4 (112)	100.0 (1.193)
AbT	0.7 (4)	0.3 (2)	1.5 (9)	-	1.0 (6)	6.3 (37)	0.3 (2)	5.2 (31)	1.7 (10)	0.5 (3)	**37.8 (224)**	-	0.2 (1)	-	1.2 (7)	23.0 (136)	0.7 (4)	1.7 (10)	1.2 (7)	0.7 (4)	0.3 (2)	0.5 (3)	-	13.9 (82)	0.5 (3)	0.8 (5)	100.0 (592)
Al	11.0 (66)	0.2 (1)	2.2 (13)	-	0.3 (2)	1.7 (10)	0.3 (2)	0.3 (2)	0.2 (1)	0.2 (1)	0.2 (1)	**68.1 (407)**	2.2 (13)	-	0.7 (4)	0.2 (1)	0.8 (5)	8.0 (48)	-	0.2 (1)	0.3 (2)	-	0.2 (1)	0.3 (2)	2.5 (15)	-	100.0 (598)
BnL	1.3 (8)	1.0 (6)	1.2 (7)	0.5 (3)	2.5 (15)	4.2 (25)	2.5 (15)	2.3 (14)	0.5 (3)	-	0.7 (4)	3.7 (22)	**48.5 (292)**	-	1.3 (8)	0.7 (4)	0.3 (2)	6.1 (37)	6.5 (39)	1.7 (10)	2.2 (13)	3.2 (19)	0.3 (2)	-	8.6 (52)	0.3 (2)	100.0 (602)
BnP	9.1 (54)	1.5 (9)	2.7 (16)	1.0 (6)	3.9 (23)	0.8 (5)	0.2 (1)	3.2 (19)	0.3 (2)	0.7 (4)	0.2 (1)	4.4 (26)	-	**50.5 (301)**	6.0 (36)	0.8 (5)	0.7 (4)	0.2 (1)	-	3.0 (18)	2.2 (13)	0.7 (4)	0.8 (5)	2.2 (13)	4.7 (28)	0.3 (2)	100.0 (596)
CbS	1.3 (8)	6.2 (37)	1.7 (10)	1.2 (7)	2.5 (15)	0.3 (2)	0.2 (1)	1.7 (10)	-	-	0.8 (5)	0.8 (5)	0.5 (3)	-	**69.5 (417)**	0.8 (5)	1.7 (10)	0.5 (3)	0.8 (5)	0.8 (5)	1.0 (6)	2.8 (17)	1.7 (10)	1.5 (9)	1.7 (10)	-	100.0 (600)
ClS	0.2 (1)	0.3 (2)	1.3 (8)	0.3 (2)	0.2 (1)	0.7 (4)	-	2.0 (12)	0.2 (1)	0.3 (2)	14.0 (84)	-	0.2 (1)	-	-	**62.7 (376)**	0.7 (4)	-	0.2 (1)	-	-	-	-	16.2 (97)	-	0.7 (4)	100.0 (600)
CuU	3.0 (18)	0.3 (2)	-	-	0.5 (3)	1.5 (9)	-	11.1 (67)	0.2 (1)	-	0.2 (1)	0.3 (2)	0.3 (2)	-	2.3 (14)	0.5 (3)	**73.9 (445)**	-	2.3 (14)	0.2 (1)	0.2 (1)	0.2 (1)	0.2 (1)	2.7 (16)	0.2 (1)	-	100.0 (602)
LcA	1.4 (8)	-	1.0 (6)	0.5 (3)	-	2.7 (16)	-	0.3 (2)	-	0.2 (1)	-	3.2 (19)	5.1 (30)	-	0.5 (3)	-	0.2 (1)	**61.2 (361)**	0.7 (4)	0.5 (3)	-	0.7 (4)	-	0.3 (2)	21.4 (126)	0.2 (1)	100.0 (590)
LgA	0.8 (5)	0.5 (3)	0.3 (2)	-	0.7 (4)	0.8 (5)	0.2 (1)	0.8 (5)	-	-	-	1.0 (6)	2.4 (14)	-	3.4 (20)	1.0 (6)	2.7 (16)	0.3 (2)	**83.8 (495)**	0.2 (1)	-	0.3 (2)	-	-	0.5 (3)	0.2 (1)	100.0 (591)
NrA	2.2 (13)	1.0 (6)	2.0 (12)	2.0 (12)	6.5 (39)	2.0 (12)	6.0 (36)	0.8 (5)	0.3 (2)	1.7 (10)	0.8 (5)	0.7 (4)	2.7 (16)	-	2.7 (16)	0.2 (1)	0.2 (1)	0.7 (4)	-	**27.6 (165)**	12.2 (73)	12.9 (77)	9.5 (57)	0.5 (3)	4.7 (28)	0.2 (1)	100.0 (598)
Nrt	2.7 (16)	1.0 (6)	1.7 (10)	1.3 (8)	4.3 (26)	4.0 (24)	8.3 (50)	-	2.0 (12)	2.0 (12)	-	-	0.3 (2)	-	1.3 (8)	-	-	-	-	8.0 (48)	**49.0 (294)**	7.0 (42)	6.7 (40)	-	0.3 (2)	-	100.0 (600)
Nrd	0.5 (3)	1.3 (8)	1.2 (7)	1.3 (8)	7.8 (47)	1.5 (9)	8.8 (53)	1.2 (7)	0.5 (3)	0.8 (5)	-	-	1.0 (6)	-	3.7 (22)	-	0.7 (4)	-	0.3 (2)	9.5 (57)	9.5 (57)	**35.8 (215)**	13.0 (78)	0.2 (1)	0.7 (4)	0.7 (4)	100.0 (600)
NrR	2.3 (14)	1.8 (11)	0.5 (3)	1.3 (8)	9.0 (54)	2.5 (15)	8.0 (48)	2.5 (15)	0.5 (3)	2.3 (14)	0.3 (2)	0.5 (3)	1.5 (9)	-	2.8 (17)	-	0.3 (2)	-	-	9.2 (55)	6.2 (37)	21.4 (128)	**24.5 (147)**	0.2 (1)	0.3 (2)	1.8 (11)	100.0 (599)
PzU	2.3 (14)	0.3 (2)	1.3 (8)	-	0.2 (1)	0.8 (5)	-	4.7 (28)	0.7 (4)	0.8 (5)	5.9 (35)	0.7 (4)	-	-	1.3 (8)	7.7 (46)	2.7 (16)	0.2 (1)	0.2 (1)	0.2 (1)	-	-	-	**68.7 (410)**	1.0 (6)	0.3 (2)	100.0 (597)
RtM	2.3 (14)	1.0 (6)	4.4 (26)	4.2 (25)	1.3 (8)	1.3 (8)	-	0.2 (1)	-	0.5 (3)	0.2 (1)	3.2 (19)	1.5 (9)	-	1.3 (8)	0.2 (1)	0.2 (1)	25.6 (153)	0.5 (3)	0.5 (3)	-	0.2 (1)	-	0.2 (1)	**51.3 (306)**	-	100.0 (597)
CnO	-	-	24.2 (146)	2.0 (12)	0.3 (2)	0.2 (1)	-	0.2 (1)	-	7.6 (46)	1.5 (9)	-	0.2 (1)	-	0.3 (2)	2.8 (17)	-	0.3 (2)	-	0.5 (3)	0.2 (1)	0.2 (1)	-	0.3 (2)	3.8 (23)	55.4 (334)	100.0 (603)
Overall																											58.0 (20.662)

Likewise, a comparison among the wine white grape cultivars was implemented to make easier the identification procedure of possible synonymy groups (Tab. 7). Nuragus (Nr), Nuragus Arrubiu (NrA), Nuragus Moscatello (Nrt), Nuragus Muscadeddu (Nrd) and Nuragus rosso rompizzolla (NrR) could be considered as belonging to the same synonymy group, as well as Cannonau bianco di Trieri (CnT), Cannonau bianco di Oliena (CnO) and Sinnidanu (Sn), confirming the achievements of Orrù et al. (2012). Also the cultivars Albacanna di Triei (AbT), Calabresa di Seulo (ClS) and Panzale di Ula (PzU) seems to belong to the same cluster. Other little synonymy group could be constituted by Bianca pelosa di Montresta (BnM) and Licronaxiu (Lx); Mizu (Mz) and Semidano (Sm); and Lacconarzu di Abbasanta (LcA) and Retagliaddu di Monti (RtM)

The dessert wine grape cultivars too were compared to identify possible synonymy groups. From the evaluation of the percentage of misattribution achieved by the comparison among the studied dessert wine black cultivars seems that two little groups could be formed. Girò scuro di Serri (Grs) and Moscato nero di Ulatirso (MnU) could be grouped in the same cluster, as well as Moscatello nero di Genuri (MnG) and Moscato nero di Sini (MnS) (Tab. 8).

Table 8 - Statistical comparisons among the analysed dessert black wine grape cultivars. In parentheses, the number of seeds.

	Gr	**GrG**	**Grs**	**MnG**	**MrS**	**MnS**	**MnU**	**Total**
Gr	**93.3% (1.122)**	1.6% (19)	0.1% (1)	1.0% (12)	1.1% (13)	2.0% (24)	1.0% (12)	100.0% (1203)
GrG	4.4% (26)	**80.9% (480)**	0.8% (5)	0.3% (2)	4.4% (26)	4.4% (26)	4.7% (28)	100.0% (593)
Grs	-	0.5% (3)	**87.1% (520)**	-	0.3% (2)	-	12.1% (72)	100.0% (597)
MnG	2.8% (34)	0.5% (6)	-	**85.3% (1.022)**	0.8% (10)	10.5% (126)	-	100.0% (1198)
MrS	2.8% (17)	6.7% (40)	-	1.8% (11)	**86.5% (518)**	2.2% (13)	-	100.0% (599)
MnS	5.3% (32)	4.0% (24)	0.2% (1)	34.8% (209)	3.0% (18)	**52.7% (316)**	-	100.0% (600)
MnU	1.5% (9)	6.2% (37)	15.2% (91)	-	-	0.2% (1)	**77.0% (461)**	100.0% (599)
Overall								**82.4% (5389)**

Using the same statistical analysis and the same evaluation procedure of the identification percentages, two large groups were recognized among the dessert wine white cultivars (Tab. 9). Aregu

biancu (ArB), Vernaccia di Escalaplano (VrE), Vernaccia di S. Rosalia (VrR), Vernaccia bidri di Villasor (VrV), Vernazza di Orosei (VrO), Granatza di Mamoiada (GzM) and Granatza Aregu di Seulo (GzS) could be considered as belonging to the same cluster, confirming and enriching the synonymy group proposed by Orrù et al. (2012b). Similarly, Moscato di Lodine (MLd), Moscato di Tempio 1 (MT1), Moscato su pinu di Mamoiada (MSP), Moscatello bianco (Mb), Moscato bianco di Generi (MbG), Moscato bianco di Sini (MbS) and Moscato di Fonni (MFn) seem forming an unique group, one more time corroboratine and enhancing one of the groups proposed by Orrù et al. (2012b). Two other little possible groups were identified among these cultivar: one constituted by Arvesiniadu (Av) and Alvaranzeniadu bianco di Bosa (AvB)) and the other by Codronisca (Cd) and Nasco (Ns).

Following the implications of the carried out classifiers and the suggestions of the historical and cultural information about the consumption aptitude of the studied grapevine cultivars, on the basis of the achieved results, two further discrimination analysis were implemented, hypothesizing some new synonymy groups. The analysed 54 bleck berry cultivars were grouped in 14 synonymy groups, leaveing eight of them out as independent cultivars (Tab. 10), confirming the six clusters proposed by Orrù et al. (2012b) (G1,G2,G3,G6,G8 and G14). Similarly, the 61 white berry cultivars were grouped in 15 synonymy groups, leaveing nine of them out as univocal cultivars (Tab. 11), confirming also in this case the six groups proposed by Orrù et al. (2012b) (G4,G5,G7,G9,G10 and G11).

Table 9. Statistical comparisons among the analysed dessert white wine grape cultivars. Percentage and number of seeds in parenthesis.

	ArB	Av	Cd	GzM	GzS	GzG	GB	Mb	MbG	MFn	MPt	Ns	RmS	VrR	VrS	AvB	MbS	MLd	MT1	MT2	MSP	VrV	VrE	VrO	Total
ArB	**56.2 (337)**	-	-	16.7 (100)	2.2 (13)	0.7 (4)	0.2 (1)	-	-	-	2.8 (17)	-	0.2 (1)	6.7 (40)	0.8 (5)	0.2 (1)	-	-	-	0.7 (4)	-	7.0 (42)	5.2 (31)	0.7 (4)	100.0 (600)
Av	-	**84.9 (1772)**	0.8 (16)	-	-	0.2 (4)	0.2 (4)	1.0 (20)	0.5 (10)	1.1 (22)	0.2 (4)	0.1 (2)	0.1 (2)	-	2.2 (46)	6.7 (140)	0.3 (6)	1.3 (28)	-	-	0.5 (10)	-	-	-	100.0 (2086)
Cd	0.2 (2)	1.3 (14)	**76.9 (803)**	0.1 (1)	-	-	3.0 (31)	0.8 (8)	0.6 (6)	-	-	15.8 (165)	0.5 (5)	-	0.1 (1)	0.1 (1)	0.6 (6)	0.1 (1)	-	-	-	-	-	-	100.0 (1044)
GzM	6.3 (76)	-	0.2 (2)	**51.9 (626)**	12.1 (146)	0.5 (6)	1.0 (12)	-	-	-	1.7 (20)	-	-	7.8 (94)	0.7 (8)	-	-	-	-	0.5 (6)	-	9.1 (110)	5.6 (68)	2.7 (32)	100.0 (1206)
GzS	7.2 (43)	-	-	20.8 (125)	**53.8 (323)**	-	2.3 (14)	-	-	-	1.7 (10)	-	-	3.0 (18)	-	-	-	-	-	3.7 (22)	-	3.3 (20)	1.2 (7)	3.0 (18)	100.0 (600)
GzG	1.2 (7)	0.3 (2)	0.3 (2)	0.2 (1)	0.3 (2)	**90.7 (546)**	-	0.2 (1)	2.5 (15)	-	1.8 (11)	-	-	-	0.5 (3)	-	0.5 (3)	0.3 (2)	-	0.3 (2)	0.5 (3)	0.3 (2)	-	-	100.0 (602)
GB	0.6 (6)	2.9 (30)	1.9 (20)	1.6 (17)	1.5 (16)	0.1 (1)	**75.3 (790)**	1.5 (16)	-	0.2 (2)	1.2 (13)	1.2 (13)	2.5 (26)	0.4 (4)	4.0 (42)	-	0.6 (6)	-	-	0.9 (9)	-	2.2 (23)	0.3 (3)	1.1 (12)	100.0 (1049)
Mb	-	3.1 (37)	2.4 (29)	0.3 (3)	-	0.5 (6)	2.5 (30)	**45.5 (545)**	6.4 (77)	9.7 (116)	0.9 (11)	0.3 (4)	-	-	0.1 (1)	0.5 (6)	10.2 (122)	10.4 (125)	3.1 (37)	0.3 (3)	3.9 (47)	-	-	-	100.0 (1199)
MbG	-	0.7 (4)	1.0 (6)	-	-	1.3 (8)	-	13.9 (84)	**39.3 (237)**	4.3 (26)	2.2 (13)	-	-	-	-	2.0 (12)	21.6 (130)	5.3 (32)	3.2 (19)	1.0 (6)	4.3 (26)	-	-	-	100.0 (603)
MFn	-	1.0 (9)	0.3 (3)	-	-	-	0.1 (1)	10.8 (97)	5.1 (46)	**44.2 (398)**	0.2 (2)	-	0.2 (2)	-	-	1.6 (14)	10.2 (92)	5.2 (47)	12.0 (108)	0.6 (5)	8.5 (77)	-	-	-	100.0 (901)
MPt	2.0 (12)	0.3 (2)	-	1.5 (9)	0.8 (5)	3.3 (20)	2.2 (13)	1.8 (11)	0.7 (4)	0.2 (1)	**57.7 (346)**	-	-	-	2.8 (17)	0.3 (2)	2.3 (14)	0.3 (2)	0.3 (2)	22.3 (134)	-	0.3 (2)	0.2 (1)	0.5 (3)	100.0 (600)
Ns	0.1 (1)	1.1 (8)	21.8 (163)	-	-	0.9 (7)	7.1 (53)	0.3 (2)	0.5 (4)	-	0.1 (1)	**66.2 (494)**	0.4 (3)	-	0.7 (5)	-	0.5 (4)	-	-	-	-	0.1 (1)	-	-	100.0 (746)
RmS	-	1.2 (7)	0.3 (2)	-	-	0.3 (2)	2.9 (17)	-	-	-	-	0.5 (3)	**89.1 (531)**	-	1.8 (11)	0.3 (2)	0.2 (1)	-	-	-	-	2.9 (17)	0.3 (2)	0.2 (1)	100.0 (596)
VrR	8.9 (53)	-	-	10.6 (63)	4.4 (26)	0.2 (1)	0.8 (5)	-	-	-	1.2 (7)	-	-	**45.1 (269)**	1.0 (6)	-	-	-	-	1.0 (6)	-	12.7 (76)	10.2 (61)	4.0 (24)	100.0 (597)
VrS	1.0 (10)	7.2 (76)	0.3 (3)	1.2 (13)	0.4 (4)	2.4 (25)	4.5 (47)	0.4 (4)	1.7 (18)	0.5 (5)	6.0 (63)	0.5 (5)	0.6 (6)	-	**64.9 (681)**	1.6 (17)	2.3 (24)	0.4 (4)	0.6 (6)	2.4 (25)	0.2 (2)	0.2 (2)	-	0.9 (9)	100.0 (1049)
AvB	0.2 (1)	25.5 (151)	0.3 (2)	-	-	0.7 (4)	-	0.2 (1)	0.3 (2)	0.2 (1)	0.3 (2)	-	0.2 (1)	-	0.5 (3)	**68.6 (406)**	1.2 (7)	1.0 (6)	0.2 (1)	0.2 (1)	0.5 (3)	-	-	-	100.0 (592)
MbS	-	-	0.5 (6)	-	-	3.5 (42)	0.3 (4)	3.8 (46)	9.8 (118)	5.0 (60)	2.5 (30)	-	-	-	-	0.5 (6)	**60.3 (722)**	5.0 (60)	2.7 (32)	1.2 (14)	4.8 (58)	-	-	-	100.0 (1198)
MLd	-	1.0 (6)	-	-	-	1.2 (7)	-	10.9 (65)	5.0 (30)	7.7 (46)	0.2 (1)	-	0.2 (1)	-	-	0.7 (4)	21.3 (127)	**37.1 (221)**	5.2 (31)	-	9.6 (57)	-	-	-	100.0 (596)
MT1	-	1.2 (7)	-	-	-	0.3 (2)	-	1.5 (9)	2.8 (17)	14.4 (86)	0.7 (4)	-	-	-	-	0.5 (3)	20.6 (123)	10.4 (62)	**37.7 (225)**	2.8 (17)	7.0 (42)	-	-	-	100.0 (597)
MT2	1.2 (7)	0.3 (2)	-	0.7 (4)	1.7 (10)	0.7 (4)	-	0.7 (4)	-	0.8 (5)	15.3 (92)	-	-	-	0.2 (1)	0.2 (1)	2.2 (13)	0.2 (1)	1.5 (9)	**74.5 (447)**	-	-	-	-	100.0 (600)
MSP	0.2 (1)	0.3 (2)	0.2 (1)	-	-	0.8 (5)	-	5.3 (32)	9.0 (54)	19.7 (118)	0.3 (2)	-	-	-	0.2 (1)	-	18.3 (110)	13.7 (82)	3.3 (20)	0.7 (4)	**28.0 (168)**	-	-	-	100.0 (600)
VrV	9.3 (110)	0.2 (2)	-	6.2 (74)	0.5 (6)	0.2 (2)	0.3 (4)	-	-	-	-	0.2 (2)	-	3.7 (44)	0.3 (4)	-	-	-	-	-	-	**69.9 (830)**	5.7 (68)	3.5 (42)	100.0 (1188)
VrE	4.9 (30)	-	-	8.2 (50)	0.8 (5)	0.7 (4)	0.8 (5)	-	-	-	0.5 (3)	-	0.3 (2)	10.2 (62)	0.5 (3)	-	-	-	-	0.2 (1)	-	25.3 (154)	**42.6 (259)**	4.9 (30)	100.0 (608)
VrO	1.5	-	-	10.7	1.1	-	1.1	-	-	-	1.9	0.4	-	11.9	3.3	-	-	-	-	-	-	8.1	3.7	**55.6**	100.0

	(4)			(29)	(3)		(3)				(5)	(1)		(32)	(9)								(22)	(10)	**(152)**	(270)
Overall																										**61.5 (19727)**

Table 10. Percentages of correct classification for the new hypothetical synonymy black cultivar groups. In parenthesis the number of seeds

	ApN	Axt	CcM	Gr	Grm	Ms	ObO	NeO	G1	G2	G3	G6	G8	G14	G22	G23	G24	G25	G26	G27	G28	G29	Total
ApN	**62.1 (740)**	2.0 (24)	-	6.1 (73)	1.4 (17)	-	-	0.2 (2)	6.8 (81)	1.1 (13)	-	0.3 (3)	2.0 (24)	2.3 (27)	6.9 (82)	1.5 (18)	2.4 (29)	0.5 (6)	2.9 (34)	0.3 (4)	0.3 (3)	1.0 (12)	100.0 (1.192)
Axt	1.2 (14)	**65.8 (782)**	-	1.0 (12)	0.7 (8)	0.3 (4)	0.3 (3)	0.4 (5)	9.2 (109)	2.4 (28)	1.1 (13)	0.1 (1)	1.7 (20)	-	0.2 (2)	4.5 (54)	3.5 (42)	0.7 (8)	2.5 (30)	0.3 (3)	3.4 (40)	0.9 (11)	100.0 (1.189)
CcM	-	.	**55.6 (331)**	-	0.2 (1)	-	-	0.2 (1)	7.9 (47)	-	1.2 (7)	5.2 (31)	0.2 (1)	7.6 (45)	3.0 (18)	0.2 (1)	0.2 (1)	8.4 (50)	1.0 (6)	2.5 (15)	-	6.7 (40)	100.0 (595)
Gr	3.5 (42)	1.7 (20)	0.1 (1)	**53.9 (649)**	4.8 (58)	-	-	0.1 (1)	0.6 (7)	1.2 (14)	4.2 (51)	2.2 (26)	0.1 (1)	4.7 (57)	2.8 (34)	4.2 (50)	3.7 (45)	0.5 (6)	5.0 (60)	0.1 (1)	0.8 (10)	5.8 (70)	100.0 (1.203)
Grm	1.2 (7)	0.2 (1)	0.2 (1)	0.8 (5)	**68.7 (413)**	-	-	-	1.2 (7)	0.8 (5)	0.3 (2)	1.5 (9)	3.3 (20)	0.8 (5)	3.3 (20)	8.3 (50)	4.3 (26)	0.3 (2)	1.3 (8)	-	-	3.3 (20)	100.0 (601)
Ms	-	-	-	-	-	**84.7 (254)**	1.7 (5)	-	4.7 (14)	0.3 (1)	5.0 (15)	0.3 (1)	-	-	-	-	-	2.0 (6)	1.0 (3)	-	-	0.3 (1)	100.0 (300)
ObO	-	0.3 (1)	-	-	-	1.0 (3)	**89.7 (269)**	-	2.0 (6)	-	0.7 (2)	-	0.7 (2)	-	-	-	-	0.3 (1)	0.3 (1)	-	5.0 (15)	-	100.0 (300)
NeO	4.2 (9)	0.5 (1)	-	-	-	-	-	**70.4 (152)**	4.2 (9)	-	2.3 (5)	0.5 (1)	0.5 (1)	1.9 (4)	-	-	0.9 (2)	3.7 (8)	-	2.8 (6)	6.5 (14)	1.9 (4)	100.0 (216)
G1	2.3 (105)	2.8 (127)	2.7 (121)	0.1 (5)	0.1 (6)	0.7 (32)	0.4 (16)	0.2 (9)	**67.5 (3.030)**	0.9 (40)	1.7 (78)	1.2 (54)	1.9 (85)	3.9 (173)	1.4 (61)	1.1 (48)	1.9 (86)	2.2 (99)	2.5 (112)	0.4 (16)	0.7 (33)	3.4 (154)	100.0 (4.490)
G2	1.2 (21)	4.6 (82)	-	0.2 (3)	0.3 (5)	0.3 (6)	-	0.4 (7)	8.6 (155)	**60.1 (1.079)**	1.2 (21)	-	2.6 (46)	1.4 (26)	0.6 (10)	6.1 (109)	6.3 (114)	0.1 (1)	0.6 (11)	-	1.2 (21)	4.4 (79)	100.0 (1.796)
G3	-	0.4 (7)	0.1 (2)	0.2 (3)	0.2 (4)	-	0.2 (4)	0.1 (1)	4.0 (72)	2.3 (42)	**65.7 (1.180)**	3.0 (53)	1.0 (18)	6.6 (118)	0.2 (4)	0.1 (2)	2.6 (47)	2.2 (40)	5.1 (91)	0.3 (5)	0.6 (10)	5.2 (93)	100.0 (1.796)
G6	0.6 (30)	-	0.2 (11)	1.4 (69)	0.2 (10)	-	-	-	2.1 (99)	-	3.1 (149)	**85.3 (4.066)**	0.1 (3)	4.2 (198)	0.8 (40)	-	0.1 (7)	0.1 (7)	0.5 (23)	0.1 (4)	-	1.0 (48)	100.0 (4.764)
G8	1.0 (21)	1.9 (40)	0.3 (6)	0.5 (11)	5.1 (107)	- (1)	-	-	7.9 (165)	6.0 (125)	-	0.3 (7)	**55.6 (1.165)**	0.6 (13)	3.6 (75)	7.2 (151)	7.2 (150)	0.8 (17)	1.0 (22)	0.2 (4)	0.2 (4)	0.6 (12)	100.0 (2.096)
G14	1.2 (37)	0.2 (6)	1.6 (48)	1.8 (53)	0.3 (8)	-	-	-	6.2 (185)	-	5.2 (157)	4.9 (146)	0.4 (12)	**64.9 (1.946)**	0.7 (20)	0.6 (18)	2.4 (71)	1.1 (34)	6.2 (187)	- (1)	- (1)	2.2 (67)	100.0 (2.997)
G22	1.9 (23)	0.3 (3)	3.4 (41)	1.1 (13)	2.3 (28)	-	0.1 (1)	0.1 (1)	4.8 (57)	0.4 (5)	1.8 (22)	5.1 (61)	0.7 (8)	4.5 (54)	**54.7 (652)**	1.8 (22)	0.4 (5)	2.9 (35)	4.0 (48)	1.4 (17)	2.3 (28)	5.8 (69)	100.0 (1.193)
G23	0.6 (10)	0.5 (9)	-	1.1 (20)	2.8 (49)	-	-	0.1 (1)	3.8 (68)	6.1 (108)	1.0 (18)	-	1.8 (32)	1.0 (17)	0.2 (4)	**73.2 (1.294)**	3.7 (65)	0.2 (3)	1.6 (29)	0.1 (2)	1.4 (24)	0.8 (15)	100.0 (1.768)
G24	1.1 (18)	3.2 (52)	0.1 (1)	3.9 (64)	1.5 (25)	-	-	-	4.5 (74)	4.2 (69)	2.4 (39)	0.2 (4)	4.0 (66)	4.2 (70)	-	5.0 (83)	**58.7 (969)**	0.3 (5)	4.9 (81)	-	-	1.8 (30)	100.0 (1.650)
G25	0.1 (1)	0.4 (7)	1.8 (32)	0.1 (2)	-	0.1 (2)	2.1 (37)	0.1 (1)	7.0 (126)	0.1 (2)	1.6 (28)	0.1 (2)	0.4 (8)	0.4 (8)	1.1 (19)	-	-	**73.2 (1.311)**	5.1 (92)	4.0 (71)	0.9 (17)	1.4 (25)	100.0 (1.791)
G26	1.1 (34)	0.2 (6)	0.2 (7)	1.5 (44)	0.2 (5)	0.1 (2)	0.1 (3)	0.1 (3)	4.4 (132)	- (1)	3.7 (111)	0.2 (6)	0.7 (21)	2.4 (72)	0.4 (13)	0.1 (4)	2.8 (82)	6.4 (191)	**71.1 (2.116)**	0.2 (7)	0.1 (2)	3.8 (112)	100.0 (2.974)
G27	0.1 (1)	-	0.6 (9)	1.1 (17)	0.2 (3)	-	0.1 (2)	0.1 (1)	0.3 (4)	-	0.9 (14)	0.1 (1)	0.1 (1)	1.3 (20)	1.3 (19)	0.2 (3)	0.3 (4)	8.2 (123)	0.1 (2)	**73.8 (1.106)**	4.5 (67)	6.8 (102)	100.0 (1.499)
G28	0.6 (12)	1.7 (35)	0.1 (3)	0.9 (18)	0.8 (17)	0.1 (3)	1.3 (26)	1.8 (37)	3.1 (62)	1.6 (33)	0.9 (19)	-	0.6 (12)	-	1.6 (32)	1.2 (24)	0.2 (4)	1.7 (34)	1.0 (20)	3.9 (78)	**72.6 (1.458)**	4.0 (81)	100.0 (2.008)
G29	2.3 (56)	0.3 (7)	2.3 (55)	1.2 (28)	0.3 (8)	-	0.2 (4)	0.4 (9)	2.9 (70)	3.6 (87)	3.2 (77)	1.4 (33)	0.3 (7)	1.2 (29)	5.3 (128)	0.2 (4)	1.7 (41)	2.3 (55)	4.8 (116)	2.0 (48)	5.1 (122)	**58.9 (1.410)**	100.0 (2.394)
Overall																							**67.9 (38.812)**

The black berry cultivars Nieddu mannu di Nurri (NNr) and Nasco nero di Abbasanta (NsA) were added to the G1 proposed by Orrù et al. (2012b) and considered synonym of Falso Gregu (FlG), Nera tomentosa (NeT), Nieddu Pedra Serra (NPS), Nieddu mannu di Pattada (NPt) and Primidivu Nieddu (PrN), reaching the 67.5% of correct identification (Tab. 10). Similarly, the cultivar Monica near (MN) was added to the G2, assumed as synonymus of Monica di Escalaplano (ME) and Monica di Seulo (MSl). For this group a performance of right classification of 60.1% was achieved (Tab. 10). Adding the cultivar Nera di Abbasanta (NeA), the group G3 shown 65.7% of correct detection (Tab. 10), although not all the cultivar analysed by Orrù et al. (2012b) were available for this work. The black berry cultivar group G6 proposed by Orrù et al. (2012b), exclusively constituted by Cannonau (Cn), was widely enriched with the cultivars Aniga di Lanusei (AnL), Cannonau nero di Sestu (CnS), Girò scuro di Serri (Grs), Moscato nero di Ulatirso (MnU) and Nera liscia di Montresta (NeL), obtaining more than 85% of correct identification (Tab. 10). Also the group G8 was enriched with a further cultivar, Cannonatu anticu di Bitti (CnB), achieving a percentage of correct recognition of 55.6%; while the group G14 remained unchanged, showing a performance of correct identification of 64.9% (Tab. 10). The new hypothetical groups from G22 to G29 shown percentages of correct identification ranged between 54.7% (G22) and 73.8% (G27) (Tab. 10). In table 11, the cultivars included in each synonymy group are reported. The cultivars Apesorgia near (ApN), Axina de tres bias (Axt), Caricagiola di Monti (CcM), Girò (Gr), Girò morbido di Serri (Grm), Mustiosa (Ms), Ocre boe di Orosei (ObO) and Nera di Orosei (NeO) were not grouped in any synonymy cluster, remaining independent cultivars, and showing correct classification performances included between 53.9% (Gr) and 89.7% (Ms). An overall cross-validated percentage of correct reconition of 67.9% was reached for the new hypotehical synonymy black cultivar groups (Tab. 10).

Regarding the white cultivar synonymy groups proposed in this work, the groups G4, G7 and G9 remained unchanged respect to the results obtained by Orrù et al. (2012b), showing percentages of correct identification included between 63.4 % (G4) and 67.6% (G9) (Tab. 12). The group G5 was amply enriched, adding the cultivars Granatza Aregu di Seulo (GzS), Granatza di Mamoiada (GzM), Vernaccia bidri di Villasor (VrV) and Vernazza di Orosei (VrO), as well as the group G10 that was enhanced with Moscatello bianco (Mb), Moscatello bianco di Sini (MbS) and Moscato di Fonni (MFn). These two groups achieved percentages of right recognition of 92.2% and 85.6%, respectively (Tab. 12). Also te white cultivar group G11 proposed by Orrù et al. (2012b), exclusively constituted by Gregu biancu

(GB), was enriched with the cultivars Bianca di Lodine (BnL), obtaining 52.8% of correct identification (Tab. 12).

Tabele 10. Percentages of correct classification for the new hypothetical synonymy black cultivar groups. In parenthesis the number of seeds.

	ApN	Axt	CcM	Gr	Grm	Ms	ObO	NeO	G1	G2	G3	G6	G8	G14	G22	G23	G24	G25	G26	G27	G28	G29	Total
ApN	**62.1 (740)**	2.0 (24)	-	6.1 (73)	1.4 (17)	-	-	0.2 (2)	6.8 (81)	1.1 (13)	-	0.3 (3)	2.0 (24)	2.3 (27)	6.9 (82)	1.5 (18)	2.4 (29)	0.5 (6)	2.9 (34)	0.3 (4)	0.3 (3)	1.0 (12)	100.0 (1.192)
Axt	1.2 (14)	**65.8 (782)**	-	1.0 (12)	0.7 (8)	0.3 (4)	0.3 (3)	0.4 (5)	9.2 (109)	2.4 (28)	1.1 (13)	0.1 (1)	1.7 (20)	-	0.2 (2)	4.5 (54)	3.5 (42)	0.7 (8)	2.5 (30)	0.3 (3)	3.4 (40)	0.9 (11)	100.0 (1.189)
CcM	-	.	**55.6 (331)**	-	0.2 (1)	-	-	0.2 (1)	7.9 (47)	-	1.2 (7)	5.2 (31)	0.2 (1)	7.6 (45)	3.0 (18)	0.2 (1)	0.2 (1)	8.4 (50)	1.0 (6)	2.5 (15)	-	6.7 (40)	100.0 (595)
Gr	3.5 (42)	1.7 (20)	0.1 (1)	**53.9 (649)**	4.8 (58)	-	-	0.1 (1)	0.6 (7)	1.2 (14)	4.2 (51)	2.2 (26)	0.1 (1)	4.7 (57)	2.8 (34)	4.2 (50)	3.7 (45)	0.5 (6)	5.0 (60)	0.1 (1)	0.8 (10)	5.8 (70)	100.0 (1.203)
Grm	1.2 (7)	0.2 (1)	0.2 (1)	0.8 (5)	**68.7 (413)**	-	-	-	1.2 (7)	0.8 (5)	0.3 (2)	1.5 (9)	3.3 (20)	0.8 (5)	3.3 (20)	8.3 (50)	4.3 (26)	0.3 (2)	1.3 (8)	-	-	3.3 (20)	100.0 (601)
Ms	-	-	-	-	-	**84.7 (254)**	1.7 (5)	-	4.7 (14)	0.3 (1)	5.0 (15)	0.3 (1)	-	-	-	-	-	2.0 (6)	1.0 (3)	-	-	0.3 (1)	100.0 (300)
ObO	-	0.3 (1)	-	-	-	1.0 (3)	**89.7 (269)**	-	2.0 (6)	-	0.7 (2)	-	0.7 (2)	-	-	-	-	0.3 (1)	0.3 (1)	-	5.0 (15)	-	100.0 (300)
NeO	4.2 (9)	0.5 (1)	-	-	-	-	-	**70.4 (152)**	4.2 (9)	-	2.3 (5)	0.5 (1)	0.5 (1)	1.9 (4)	-	-	0.9 (2)	3.7 (8)	-	2.8 (6)	6.5 (14)	1.9 (4)	100.0 (216)
G1	2.3 (105)	2.8 (127)	2.7 (121)	0.1 (5)	0.1 (6)	0.7 (32)	0.4 (16)	0.2 (9)	**67.5 (3.030)**	0.9 (40)	1.7 (78)	1.2 (54)	1.9 (85)	3.9 (173)	1.4 (61)	1.1 (48)	1.9 (86)	2.2 (99)	2.5 (112)	0.4 (16)	0.7 (33)	3.4 (154)	100.0 (4.490)
G2	1.2 (21)	4.6 (82)	-	0.2 (3)	0.3 (5)	0.3 (6)	-	0.4 (7)	8.6 (155)	**60.1 (1.079)**	1.2 (21)	-	2.6 (46)	1.4 (26)	0.6 (10)	6.1 (109)	6.3 (114)	0.1 (1)	0.6 (11)	-	1.2 (21)	4.4 (79)	100.0 (1.796)
G3	-	0.4 (7)	0.1 (2)	0.2 (3)	0.2 (4)	-	0.2 (4)	0.1 (1)	4.0 (72)	2.3 (42)	**65.7 (1.180)**	3.0 (53)	1.0 (18)	6.6 (118)	0.2 (4)	0.1 (2)	2.6 (47)	2.2 (40)	5.1 (91)	0.3 (5)	0.6 (10)	5.2 (93)	100.0 (1.796)
G6	0.6 (30)	-	0.2 (11)	1.4 (69)	0.2 (10)	-	-	-	2.1 (99)	-	3.1 (149)	**85.3 (4.066)**	0.1 (3)	4.2 (198)	0.8 (40)	-	0.1 (7)	0.1 (7)	0.5 (23)	0.1 (4)	-	1.0 (48)	100.0 (4.764)
G8	1.0 (21)	1.9 (40)	0.3 (6)	0.5 (11)	5.1 (107)	- (1)	-	-	7.9 (165)	6.0 (125)	-	0.3 (7)	**55.6 (1.165)**	0.6 (13)	3.6 (75)	7.2 (151)	7.2 (150)	0.8 (17)	1.0 (22)	0.2 (4)	0.2 (4)	0.6 (12)	100.0 (2.096)
G14	1.2 (37)	0.2 (6)	1.6 (48)	1.8 (53)	0.3 (8)	-	-	-	6.2 (185)	-	5.2 (157)	4.9 (146)	0.4 (12)	**64.9 (1.946)**	0.7 (20)	0.6 (18)	2.4 (71)	1.1 (34)	6.2 (187)	- (1)	- (1)	2.2 (67)	100.0 (2.997)
G22	1.9 (23)	0.3 (3)	3.4 (41)	1.1 (13)	2.3 (28)	-	0.1 (1)	0.1 (1)	4.8 (57)	0.4 (5)	1.8 (22)	5.1 (61)	0.7 (8)	4.5 (54)	**54.7 (652)**	1.8 (22)	0.4 (5)	2.9 (35)	4.0 (48)	1.4 (17)	2.3 (28)	5.8 (69)	100.0 (1.193)
G23	0.6 (10)	0.5 (9)	-	1.1 (20)	2.8 (49)	-	-	0.1 (1)	3.8 (68)	6.1 (108)	1.0 (18)	-	1.8 (32)	1.0 (17)	0.2 (4)	**73.2 (1.294)**	3.7 (65)	0.2 (3)	1.6 (29)	0.1 (2)	1.4 (24)	0.8 (15)	100.0 (1.768)
G24	1.1 (18)	3.2 (52)	0.1 (1)	3.9 (64)	1.5 (25)	-	-	-	4.5 (74)	4.2 (69)	2.4 (39)	0.2 (4)	4.0 (66)	4.2 (70)	-	5.0 (83)	**58.7 (969)**	0.3 (5)	4.9 (81)	-	-	1.8 (30)	100.0 (1.650)
G25	0.1 (1)	0.4 (7)	1.8 (32)	0.1 (2)	-	0.1 (2)	2.1 (37)	0.1 (1)	7.0 (126)	0.1 (2)	1.6 (28)	0.1 (2)	0.4 (8)	0.4 (8)	1.1 (19)	-	-	**73.2 (1.311)**	5.1 (92)	4.0 (71)	0.9 (17)	1.4 (25)	100.0 (1.791)
G26	1.1 (34)	0.2 (6)	0.2 (7)	1.5 (44)	0.2 (5)	0.1 (2)	0.1 (3)	0.1 (3)	4.4 (132)	- (1)	3.7 (111)	0.2 (6)	0.7 (21)	2.4 (72)	0.4 (13)	0.1 (4)	2.8 (82)	6.4 (191)	**71.1 (2.116)**	0.2 (7)	0.1 (2)	3.8 (112)	100.0 (2.974)
G27	0.1 (1)	-	0.6 (9)	1.1 (17)	0.2 (3)	-	0.1 (2)	0.1 (1)	0.3 (4)	-	0.9 (14)	0.1 (1)	0.1 (1)	1.3 (20)	1.3 (19)	0.2 (3)	0.3 (4)	8.2 (123)	0.1 (2)	**73.8 (1.106)**	4.5 (67)	6.8 (102)	100.0 (1.499)
G28	0.6 (12)	1.7 (35)	0.1 (3)	0.9 (18)	0.8 (17)	0.1 (3)	1.3 (26)	1.8 (37)	3.1 (62)	1.6 (33)	0.9 (19)	-	0.6 (12)	-	1.6 (32)	1.2 (24)	0.2 (4)	1.7 (34)	1.0 (20)	3.9 (78)	**72.6 (1.458)**	4.0 (81)	100.0 (2.008)
G29	2.3 (56)	0.3 (7)	2.3 (55)	1.2 (28)	0.3 (8)	-	0.2 (4)	0.4 (9)	2.9 (70)	3.6 (87)	3.2 (77)	1.4 (33)	0.3 (7)	1.2 (29)	5.3 (128)	0.2 (4)	1.7 (41)	2.3 (55)	4.8 (116)	2.0 (48)	5.1 (122)	**58.9 (1.410)**	100.0 (2.394)
Overall																							**67.9 (38.812)**

Table 12. Percentages of correct classification for the new hypothetical synonymy white cultivar groups. In parenthesis the number of seeds.

	AxF	Cob	GzG	RmS	AGA	Al	BnP	CbS	PsN	G4	G5	G7	G9	G10	G11	G12	G13	G15	G16	G17	G18	G19	G20	G21	Total
AxF	**57.5 (690)**	1,0 (12)	-	0.3 (4)	0.5 (6)	3.4 (41)	-	0.3 (4)	1.1 (13)	0.7 (8)	0.2 (2)	2.8 (34)	0.2 (2)	4.3 (52)	2.1 (25)	0.4 (5)	7.2 (86)	6.2 (74)	0.8 (9)	5.7 (68)	-	-	4.8 (57)	0.6 (7)	100.0 (1199)
Cob	1.6 (17)	**77.1 (808)**	-	0.1 (1)	0.6 (6)	1.0 (10)	-	1.0 (11)	-	1.0 (10)	0.2 (2)	0.1 (1)	0.2 (2)	-	3.3 (35)	2.3 (24)	1.1 (12)	1.8 (19)	1.1 (12)	1.3 (14)	4.8 (50)	0.2 (2)	0.1 (1)	1.0 (11)	100.0 (1048)
GzG	-	-	**79.2 (477)**	-	-	-	-	1.5 (9)	0.2 (1)	3.0 (18)	3.2 (19)	6.1 (37)	1.0 (6)	2.0 (12)	-	-	-	0.7 (4)	0.2 (1)	2.2 (13)	-	-	0.2 (1)	0.7 (4)	100.0 (602)
RmS	0.5 (3)	-	-	**77.0 (459)**	0.2 (1)	1.7 (10)	-	-	6.9 (41)	0.8 (5)	1.7 (10)	-	-	-	0.7 (4)	-	1.8 (11)	4.5 (27)	2.9 (17)	0.2 (1)	0.2 (1)	-	1.0 (6)	-	100.0 (596)
AGA	2.2 (13)	0.3 (2)	0.7 (4)	-	**61.0 (366)**	1.8 (11)	-	2.7 (16)	-	1.0 (6)	0.5 (3)	-	0.5 (3)	0.2 (1)	1.2 (7)	2.3 (14)	4.3 (26)	2.8 (17)	1.3 (8)	5.7 (34)	7.3 (44)	-	0.3 (2)	3.8 (23)	100.0 (600)
Al	8.5 (51)	1.2 (7)	-	0.7 (4)	1.0 (6)	**61.2 (366)**	-	-	2.3 (14)	1.0 (6)	2.2 (13)	5.2 (31)	0.3 (2)	1.7 (10)	4.3 (26)	-	1.2 (7)	0.8 (5)	6.7 (40)	0.8 (5)	-	-	0.5 (3)	0.3 (2)	100.0 (598)
BnP	4.5 (27)	-	1.7 (10)	-	0.8 (5)	1.7 (10)	**50.5 (301)**	1.0 (6)	0.8 (5)	4.7 (28)	0.2 (1)	7.7 (46)	1.8 (11)	9.4 (56)	-	-	2.9 (17)	1.0 (6)	1.5 (9)	2.9 (17)	-	-	4.0 (24)	2.9 (17)	100.0 (596)
CbS	0.3 (2)		0.7 (4)	-	1.2 (7)	0.3 (2)	-	**61.5 (369)**	0.2 (1)	9.2 (55)	0.7 (4)	1.8 (11)	2.0 (12)	4.0 (24)	0.5 (3)	0.2 (1)	4.3 (26)	0.5 (3)	1.3 (8)	4.2 (25)	0.5 (3)	-	0.3 (2)	6.3 (38)	100.0 (600)
PsN	2.5 (15)	-	-	3.2 (19)	0.2 (1)	3.5 (21)	-	0.5 (3)	**65.9 (395)**	0.2 (1)	1.2 (7)	2.0 (12)	-	-	4.2 (25)	-	0.7 (4)	2.5 (15)	5.5 (33)	2.8 (17)	-	-	5.2 (31)	-	100.0 (599)
G4	0.9 (29)	0.1 (3)	1.2 (40)	0.1 (2)	0.5 (16)	0.2 (6)	-	0.7 (22)	0.1 (2)	**63.4 (2083)**	2.5 (83)	2.7 (88)	2.6 (87)	9.6 (316)	3.2 (105)	0.8 (27)	1.4 (45)	2.8 (92)	0.8 (26)	0.7 (22)	0.7 (24)	-	0.4 (14)	4.7 (153)	100.0 (3285)
G5	0.1 (3)		0.4 (18)	-	-	0.1 (4)	-	- (1)	- (1)	1.2 (63)	**92.2 (4673)**	0.6 (28)	2.0 (100)	-	0.9 (46)	- (2)	-	- (1)	2.0 (99)	0.3 (16)	0.1 (3)	-	-	0.2 (11)	100.0 (5069)
G7	3.4 (139)	- (2)	0.4 (16)	0.4 (17)	0.1 (3)	0.7 (30)	-	0.4 (15)	1.3 (52)	2.1 (83)	0.7 (30)	**63.6 (2573)**	2.3 (95)	7.9 (320)	0.5 (21)	0.4 (17)	0.7 (28)	1.3 (52)	3.5 (143)	4.9 (198)	0.2 (9)	-	4.5 (182)	0.5 (20)	100.0 (4045)
G9	0.1 (1)	-	0.9 (17)	-	-	0.3 (6)	-	1.3 (23)		5.8 (104)	4.1 (74)	8.4 (151)	**67.6 (1217)**	3.1 (55)	0.8 (15)	-	0.1 (1)	0.1 (1)	1.1 (20)	0.6 (11)	-	-	-	5.8 (105)	100.0 (1801)
G10	0.4 (21)	-	1.2 (70)	0.1 (3)	0.1\ (8)	0.2 (13)	-	0.6 (32)	0.1 (5)	2.5 (145)	0.1 (4)	1.6 (92)	1.3 (72)	**85.6 (4876)**	0.1 (7)	0.5 (31)	0.8 (47)	1.8 (103)	0.1 (3)	0.3 (19)	0.1 (3)	-	0.9 (53)	1.5 (87)	100.0 (5694)
G11	2.8 (47)	1.1 (18)	-	1.0 (16)	0.4 (7)	1.2 (20)	-	0.3 (5)	1.0 (17)	8.8 (145)	5.3 (88)	2.4 (39)	0.7 (12)	0.8 (13)	**52.8 (872)**	0.8 (13)	1.0 (17)	6.1 (101)	4.4 (73)	1.3 (21)	6.1 (100)	-	0.2 (3)	1.5 (24)	100.0 (1651)
G12	2.0 (36)	1.3 (23)	-	0.2 (3)	1.1 (19)	0.3 (5)	-	0.1 (2)	-	1.8 (33)	0.2 (4)	0.3 (5)	-	0.5 (9)	1.3 (23)	**68.4 (1224)**	5.4 (96)	9.3 (166)	0.2 (3)	5.7 (102)	1.6 (29)	-	0.2 (3)	0.3 (5)	100.0 (1790)
G13	2.0 (36)	0.3 (5)	-	-	0.8 (15)	0.2 (4)	-	0.7 (13)	0.4 (8)	1.4 (26)	-	2.1 (39)	-	1.0 (18)	1.8 (33)	5.4 (99)	**65.7 (1203)**	6.5 (119)	0.2 (4)	7.8 (143)	1.3 (24)	0.1 (1)	1.7 (32)	0.4 (8)	100.0 (1830)
G15	2.9 (90)	-	0.4 (14)	2.4 (74)	2.6 (81)	1.1 (35)	-	0.9 (28)	1.1 (35)	6.3 (197)	1.7 (53)	1.8 (58)	0.2 (7)	0.9 (28)	1.8 (58)	5.7 (178)	5.4 (170)	**53.9 (1696)**	0.6 (18)	3.4 (107)	2.2 (68)	-	4.4 (139)	0.4 (13)	100.0 (3147)
G16	1.1 (13)	0.5 (6)	0.3 (4)	1.5 (18)	0.7 (8)	1.7 (20)	-	0.6 (7)	1.5 (18)	0.8 (10)	8.8 (104)	5.1 (61)	7.5 (89)	0.3 (4)	6.2 (74)	-	0.3 (4)	1.4 (17)	**59.0 (700)**	1.2 (14)	0.2 (2)	-	0.9 (11)	0.3 (3)	100.0 (1187)
G17	0.5 (21)	-	-	-	0.2 (9)	0.1 (4)	-	0.1 (4)	0.5 (21)	0.3 (12)	0.5 (19)	2.7 (115)	- (1)	0.2 (10)	0.2 (7)	1.3 (53)	3.2 (135)	2.7 (115)	0.3 (13)	**85.4 (3595)**	0.5 (21)	-	1.1 (47)	0.2 (9)	100.0 (4211)
G18	0.7 (8)	9.8 (116)	-	0.2 (2)	3.3 (39)	1.1 (13)	-	1.1 (13)	0.3 (3)	0.9 (11)	0.1 (1)	0.8 (10)	0.3 (3)	0.1 (1)	4.2 (50)	1.4 (17)	2.5 (30)	0.2 (2)	1.2 (14)	2.0 (24)	**69.4 (824)**	-	-	0.6 (7)	100.0 (1188)
G19	-	5.6 (65)	-	-	1.8 (21)	-	-	-	-	-	-	-	-	-	-	-	2.5 (29)	-	-	-	0.4 (5)	**88.9 (1030)**	-	0.8 (9)	100.0 (1159)
G20	1.9 (51)	-	- (1)	-	0.1 (2)	0.4 (11)	-	0.1 (2)	0.7 (20)	0.3 (8)	-	7.3 (195)	- (1)	4.1 (110)	-	0.1 (4)	1.0 (27)	5.9 (159)	0.5 (13)	2.2 (60)	-	-	**75.1 (2012)**	0.1 (2)	100.0 (2678)
G21	0.9 (18)	0.1 (1)	0.8 (16)	-	1.9 (37)	0.3 (5)	-	3.2 (62)	-	8.8 (171)	1.6 (31)	1.4 (28)	4.8 (94)	5.4 (104)	1.1 (21)	0.2 (4)	1.1 (22)	0.5 (10)	0.3 (5)	1.4 (28)	0.4 (7)	-	-	**65.8 (1277)**	100.0 (1941)

Overall	**72.3 (47114)**

The new hypothetical groups G12, G13 and from G15 to G21 shown percentages of correct identification ranged between 53.9% (G15) and 88.9% (G19) (Tab. 12). Table 11 shows the cultivars included in each synonymy group. The cultivars Axina de Francia (AxF), Corniola bianca (Cob), Granazza di Garaumele (GzG), Remugiau di Serri (RmS), Aghina de Gerusalemme di Abbasanta (AGA), Alicante (Al), Bianca di Padria (BnP), Caddiu biancu di Serri (CbS) and Pascale di nurri (PsN) did not show no similarity with the proposed groups, remaining independent cultivars. These cultivars shown percentages of correct identification ranged between 50.5% (BnP) and 79.2% (GzG). The overall cross-validated percentage of correct classification was 72.3% for the new hypotehical synonymy white cultivar groups (Tab. 12).

Table 11. New hypothetical synonymy groups achieved on the basis of morpho-colorimetrical data. In bold the groups previously proposed by Orrù et al. 2012 and here confirmed.

New Synonymy groups	Grape variety
G1	**FlG** - **NeT** - NNr - **NPS** - **NPt** - NsA - **PrN**
G2	**ME** - MN - **MSl**
G3	**MSr** - NeA
G4	**Nr** - **NrA** - **Nrt** - **Nrd** - **NrR**
G5	**ArB** - GzS - GzM - VrV - **VrE** - **VrR** - VrO
G6	AnL - **Cn** - CnS - Grs - MnU - NeL
G7	**CnO** - **CnT** - **Sn** - **VrS**
G8	**NeB** - **NPl** - CnB
G9	**Cl** - **MPt** - **MT2**
G10	Mb - MbS - MFn - **MLd** - **MT1** - **MSP**
G11	BnL - **GB**
G12	Cd - Ns
G13	CuU - MB
G14	**GNC** - **GNS** - **Vrt**
G15	Mz - Sm
G16	LcA - RtM
G17	AbT - ClS - CfO - GlE - GlN - PzU - TtM
G18	LgA - OlT
G19	TtG - TtS
G20	AvB - Av
G21	BnC - BnM - Lx
G22	GrG - LxN
G23	AcA - NeS - SpS
G24	BrS - NeE
G25	FdS - TrS
G26	Cs - MuS - Mu
G27	CgN - CrT - FlC
G28	MnG - MnS - NeJ
G29	MrS - NeM - NSl - NrS

6.5. Conclusion

As discussed above, Sardinia is characterized by a huge number of cultivars (Grassi et al. 2003, 2008, Lovicu et al. 2010). Some of these surely derive from different breeding events involving both local and out-coming material, as well as both domesticated and wild grapes, but despite the large and complex grapevine survey, a lot of Sardinian varieties simply are the product of linguistic distorting due to the wide heterogeneity historic-cultural of the island. This phenomena generated a great assortment of grape names, that, together with the huge real number of cultivars, is the cause of the incredible current grapevine Sardinian panorama (De Mattia et al. 2007).

Many times image analysis technology proved to be able to screen biodiversity phenotyping and comparing different plant cultivars, allowing direct inferences about morphological and genetical diversity and interrelationships among organisms at different taxonomical levels without the confounding environmental effects (Bacchetta et al. 2008; 2011; Grillo et al. 2010; 2012; Mattana et al. 2008). Considering the convinceing results achived with the synonymy study of grapevine cultivars conducted by Orrù et al. (2012b) on the basis of 113 morpho-colorimetric features compared with the previous SSR analysis conducted by De Mattia et al. (2007) on the same material, in this work the same seed morpho-colorimetric features and EFDs obtained by image analysis were used to implement dedicated statistical classifiers able to discriminate among to the studied cultivars of grapewine, also in view of specific aspects as grape colour and consumption aptitude.

Notwithstanding the seeming incongruences related to the aptitude of some cultivars belonging to the same synonymy groups, due to the fact that probably same grapevine cultivars, cropped in different time and/or in different areas and maybe appling different coltural practices, can show variable berry morphology, historically assuming different uses and aptitudes in different regional areas, the achieved results seems to be consistent to the

geographical distribution of the grapevine cultivar and to the historical and cultural knowledge and are in perfect harmony with the achievements of Orrù et al. (2012b).

6.6. References

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7. Conclusions

The main conclusions achieved in the present doctoral thesis are summarized in the following points:

1. In thermal niche object of this study, fresh seeds of *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel.) Hegi of the four investigated Sardinian populations were physiologically dormant at dispersal. Cold stratification released seed dormancy suggesting spring emergence for this species. Cold environments led to slightly immature seeds at the time of seed harvest for the two high-altitude (> 700 m a.s.l.) populations. The lowest temperature range for germination (ca. 9-10.5°C) and the thermal time requirements observed in this study identified the thermal niche for seed germination and plantlet emergence to be predicted on the basis of environmental heat sum. This approach confirmed the germination period ranging from February to May for the investigated populations, although this germination pattern should be confirmed by field germination experiments. The germination phenology of this taxon limits its distribution, in Sardinia (and in the Mediterranean region more in general), along riverbanks or colluvial sites of hilly humid slopes (Ocete et al., 2008) where seedlings can grow even during the dry summer conditions and suggesting a phenotypic adaptation to slightly different microclimates.

2. The development of a system of statistical identification, based on morpho-colorimetric and Fourier descriptors through image analysis techniques and capable to identify, classify the studied cultivars of *Vitis vinifera* L. subsp. *vinifera* and to compare them with some wild populations of *Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel) Hegi and some archaeological remains of seeds founded in Sardinia.

3. On the basis of morphological features and Elliptic Fourier Descriptors the relationship between the seed lots from the archaeological sites of Sa Osa and Isola di Coltellazzo, modern cultivars historically grown close to the archaeological sites and the wild populations of *V. v. sylvestris* collected near these two sites was characterized . In particular, the analysis of the archaeological seeds of *Vitis* L. from the eight stratigraphic units of the shaft N of pre-Nuragic and Nuragic complex of Sa Osa has shown that the seeds are very similar, confirming that the different stratigraphic units belong to the same period (Middle and Final Bronze Age).

The higher similarity of the archaeological seeds to *V. v. vinifera* cultivars compared with that of *V. v. sylvestris* populations, and especially to white grapes rather than black grape cultivars, could prove that in the Campidano region in southern Sardinia white grapes probably were already used in 1600-1200 B.C. This finding may explain that white grapes, named "Vernaccia", are still traditionally cropped today in the Campidano region to produce famed wines not only by chance.

4. The synonymy study of grapevine cultivars on the 113 measurements (80 morpho-colorimetric and 33 on Fourier descriptors) features provided enough insight to achieve a clear discrimination among the synonymy groups, as confirmed by the previous SSR analysis conducted in a precedent study on the same material .

5. Seed morpho-colorimetric features and EFDs obtained by image analysis were used to implement dedicated statistical classifiers capable to discriminate among the studied cultivars of grapevine, also in view of specific aspects as grape colour and consumption aptitude.

The outward incongruences related to the aptitude to the consumption or winemaking of some cultivars belonging to the same synonymy groups were due to the fact that probably the same grapevine cultivars, cropped in different time and/or in different areas and through different coltural practices, can show even high variable berry morphology. Assuming different uses in different regional areas, the achieved results seems to be consistent to the geographical distribution of the grapevine cultivar and to the historical and cultural knowledge. Moreover, these results are in perfect harmony with the achievements of previous studies carried out on the synonymy of some grapevine cultivars.

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