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Wild grapevines of Georgia and their relationship with local cultivars: a Bayesian analysis of seed morphology

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Summary

The wild grapevines of Georgia and the South Caucasus in general are of great interest because of their relationship to the history of grapevine cultivation. Grape seeds provide information of interest about the characteristics and origin of the plant that produces them. The use of classical morphometric techniques, together with the use of combined domestication indices, multivariate analysis and Bayesian inference, applied to the study of grape seeds, have made it possible to detect a high level of domestication in the wild populations analyzed, possibly due to the presence of feral individuals and others of hybrid origin. This may pose a problem for the conservation of local autochthonous populations of wild grapevine and at the same time makes these mixed populations a reservoir of genes of interest for the improvement of cultivated grapevine or the recovery of ancient varieties nowadays predisposed in cultivation.

Keywords

Ampelography, grapevine, biodiversity, genetic resources, agrobiodiversity, *Vitis vinifera*, Caucasus, feral, hybrid

Introduction

The South Caucasus region is located between the Black and Caspian Seas and extends over several countries, notably Armenia, Azerbaijan and Georgia, being an important refuge area for numerous wild relatives of cultivated fruit species such as sweet chestnut, walnut and grapevine (Aradhya *et al.*, 2017; Huglin and Schneider, 1986; Krebs *et al.*, 2019; Ramishvili, 1988; Ramishvili, 2001; Vavilov, 1931).

Vitis (Vitaceae) includes over 70 species, which extend in the northern hemisphere across North America, Europe, North Africa and Asia. *Vitis vinifera* L. is traditionally grown from Central Asia and the South Caucasus zone to a wide range of Western Europe and the Mediterranean basin (Arnold *et al.*, 1998). *Vitis vinifera* was also introduced in distant territories such as Australia, South America and South Africa. The Caucasus houses an extremely high grapevine diversity, both wild and cultivated (Haxthausen, 1856; Kolenati, 1846; Negrul, 1938; Vavilov, 1926) and it is part of the grapevine's "Fertile Triangle" or "Vavilov´s Triangle" (Robinson *et al.*, 2013). The wild grapevine of the Caucasus was a widespread plant in Georgia but after the invasion of phylloxera and fungal diseases in the 19th century the number of plants sharply decreased. However, in Georgia the typical wild *Vitis sylvestris* C.C. Gmel. ("Usurvazi", "Krikina", "Tkisvazi" in Georgian) is still found, described and protected (Maghradze, 2008).

The South Caucasus region has been postulated as the cradle of viticulture and winemaking (McGovern, 2003; 2004, McGovern *et al.*, 2017; McGovern *et al.*, 1995; Zohary and Hopf, 2000). Results of archaeological studies of the Shulaveris group (Dangreuli Gora, Imiri, Gadachrili Gora) indicate the high level of viticulture and winemaking development in southern Georgia in the 6th to the 4th millennium B.C (Glonti, 2010). Cultural layers of the Shulaveris-Gora settlements, where pips of cultural *Vitis vinifera* were found, were 14C dated to 7942, 7624 and 7607 calendar years before present (Kvavadze *et al.*, 2007). By the end of the 2nd millennium B.C and the beginning of the 1st millennium B.C., a huge progress was made in terms of grape cultivation proved by the discovery of bronze vine cutting knifes, hatchets and other tools. Kolkhetian wines were mentioned by Homer in the "Iliad" (Glonti 2010). The finding of McGovern *et al*, (2017) pointed

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to Georgia in the South Caucasus as an initial point of European winemaking.

Based on the wild grape's selection and due to the ancient history of viticulture and winemaking, Georgians created a large diversity of autochthonous varieties with diverse agronomical and enological aptitudes: the number of the local varieties is ca. 600 (Ketskhoveli *et al.*, 1960; Ujmajuridze *et al.* 2018). They originated in the different provinces of the country, namely: Kakheti, Kartli, Imereti, Racha, Lechkhumi, Samegrelo, Guria, Adjara, Abkhazeti (Ramishvili, 2001). The varieties were described by the methods of ampelography (Staroselskii, 1893; Cholokashvili, 1939; Ketskhoveli *et al.*, 1960; Del Zan *et al.*, 2009; Maghradze *et al.*, 2012, 2017), molecular genetics (Imazio *et al.*, 2013; De Lorenzis *et al.*, 2015a) and their enological qualities were recorded (Ketskhoveli *et al.*, 1960).

The selection of specific phenotypes characterized the transition from wild grapevine to cultivars. This process implies changes within the reproductive system, which guarantees a high productive grade, together with drastic changes in the grape dimensions, its sugar and acidity content, the bunch shape and dimensions, the number and shape of seeds and a more attractive color (Rivera and Walker, 1989; Jacquat and Martinoli, 1999; Zohary and Hopf, 2000, Arroyo-García *et al.,* 2006; This *et al.* 2006, Rivera *et al.*, 2007; Maghradze *et al.* 2021). *Vitis vinifera* L. cultivars are classified according to the final product for which they have been selected, into wine grapes, table grapes and raisins. In fact, numerous *V. vinifera* cultivars produce table grapes, and, much more, grapes for wine production. The biogeographical groups of grapevine cultivars were defined by A.M. Negrul (1938, 1946), and later by Troshin *et al.* (1990).

Dioecious *Vitis sylvestris* C.C. Gmelin (*V. vinifera* subsp. *sylvestris* (C.C. Gmelin) Hegi) is supposed to be the ancestor of hermaphrodite cultivated grapevine (Crespan, 2004; Rossetto *et al.*, 2002; Sefc *et al.*, 2003; This *et al.*, 2004; Zohary and Hopf, 2000). Wild grapevine is a typical representative of the South Caucasian flora. It grows sporadically in woods, forests, lowlands and riverbanks up to 1,200 m a.s.l (Ocete *et al.*, 2018). Eyriés (1841) indicated that grapevine grows in the gullies and plains of South Caucasus as in their primitive homeland. *Smilax, Hedera, Vitis*, and *Lonicer*a are abundant lianas in the narrow species-rich forest edge along the lowland waterways passing through the Pichora system of Georgia. Wild grapevine shows some correlation with *Pterocarya fraxinifolia*, *Diospyros lotus* or *Quercus hartwisiana* forests (Denk *et al.* 2001; Ocete *et al.*, 2018). Some other wild *Vitis* species of American origin such as *Vitis riparia*, *Vitis rupestris* or *Vitis berlandieri*, were introduced in Eurasia during the 19th century and are used as rootstock due to their resistance against Phylloxera or were used in the first breeding programs to obtain varieties resistant to grapevine downy mildew. These rootstocks or their hybrids with Eurasian grapevines became often feral in natural and semi-natural habitats and still persist there (Laguna 2003, 2004, 2006). This is a growing problem throughout the range of cultivated *Vitis vinifera*, due to the abandonment of some cultural practices, or even the total abandonment of cultivation, as is often done in the European Union, due to legal incentives (Calafat-Marzal *et al.* 2023; Wyler *et al.* 2023).

Pallas (1799–1801), a German naturalist at the service of Empress Catherine II of Russia, reported the presence of countless wild grapevine populations in South Caucasus. There were several individuals with large stems, some of them with the thickness of a ship's mast; their branches climbed the surrounding trees. Bunches of grapes were harvested by the inhabitants of the region, sometimes, when the entire grape became raisin after winter frost, in the spring season. The history of the wild grapevine in Georgia is divided in two periods: first until the mid-19th century and, second, since the 1860s until nowadays, when Oidium, Plasmopara and Phylloxera, together with industrial and urban expansion, destroyed spontaneous development of wild grapevine populations. In the early 21st century fifty Georgian populations of wild grapevine were analyzed, whose sizes varied from 1 to 20 plants (mean c. 4). According to the number of plants the populations were classified as "Very bad" (64 %) or "Bad" (24 %) and only 12 % as "Regular" Despite being so widespread in the past, *V. sylvestris* is now included in the "Red Book of Georgia" since 1982 for *in-situ* preservation. Only few populations are available in the protected areas of Georgia and the activities for preservation of *V. sylvestris* in other areas are not satisfactory (Chkhartishvili and Maghradze, 2012).

The close association of Georgian wild grapevines with Georgian cultivated accessions strongly supports their involvement in the initial domestication of grapevine (Riaz *et al.*, 2018).

Kolenati (1846) recognized, based on the indumentum of the abaxial surface of leaves, at least, two types of wild grapevine in the Caucasus:

- *Vitis sylvestris* subsp. subsp. *anebophylla* (Kolen.) Vassilcz. Published as *V. vinifera* var. *anebophylla* Kolen., in page 286-7 with long-petiolate leaves, over three inches long, glabrous or with a tomentose or araneous felt. The cluster is lax, and the habit of this grapevine is slender, and due to widely spaced branches, less crowded but more entwined.
- *Vitis sylvestris* subsp. *trichophylla* (Kolen.) Vassilcz. Published as *V. vinifera* var. *trichophylla* Kolen., in page 287 with short-petiolate leaves, rarely over two inches long, with short, straight, setose hairs on the abaxial surface, especially on the leaf veins, which are most closely connected to the epidermis. The cluster is dense, and the entire habit is compact.

In parallel, Kolenati (1846): 288, 294, 314) classifies the cultivated vineyards of South Caucasus, following their origin from either one or another of the above two wild sorts, into *V. vinifera* var. *anebophylla* Kolen., (pages 294-314) and *V. vinifera* var. *trichophylla* Kolen., (pages 314-335). It is important to mention that POWO (2024) attributes variety rank to both taxa, but Kolenati (1846) worried about their likely status as subspecies and repeatedly uses the German term "Unterart" when mentioning them, which must be translated by subspecies or even he used directly the Latin term "subspecies".

Georgia became even more relevant for understanding wild grapevine diversity after the choice of a neotype for *Vitis sylvestris* by Ferrer-Gallego *et al.* (2019) who designated the specimen collected in Georgia (Alazani River basin, Jumaskure, 41°21.588′ N, 46°35.934′ E) by Ia Pipia, which is preserved in the Herbarium of the Institute of Botany, Ilia State University (TBI barcode TBI1052417!).

Molecular studies point to the possibility that the Caucasian grapevines and those of Western Europe and the Mediterranean have different wild ancestors and relatives, with the domesticated grapevines in the South Caucasus being older (Arroyo-García *et al.,* 2006). The grapevine cultivation and domestication seem to have occurred between the 7th and 4th millennium B.C., in an area comprised between the Black Sea and Iran, notably South Caucasus (Châtaignier, 1995; Valera *et al.*, 2022 and 2023; Zohary and Hopf, 2000).

Several names circulate for those South Caucasus wild grapevines such as *Vitis caucasica* Vavilov or *V. trichophylla* (Kolen.) Vass., however an alternative view is to include all of them within a highly polymorphic *V. sylvestris* (Sosnovszky, 1974). Ramishvili investigated wild grapevines of Georgia in the second half of the 20th century (1956-1988). According to R. Ramishvili (1988), there are three types of wild grapevines in Georgia: 1. true *V. sylvestris*; 2. feral populations of *V. vinifera* cultivars; and 3. intermediate forms between these two types, named as "*V. vinifera* ssp. *silvesatis* Ram."

Leaf length, which varies from 4 to over 15 cm, allow to distinguish four wild grapevine types but leaf hairy cover discriminate two types: *typica* Negr. (leaves with hairs) and *aberrans* Negr. (without hairs) (Musayev, 2014). However, Ekhvaia and Akhalkatsi (2010), who described on morphological grounds three somewhat overlapping groups of wild grapevines (West, South and East Georgian), remark that different types of indumenta can occur in the same population and in very different combinations with the rest of characters. This variability had already been described by Kolenati (1846).

Vitis vinifera seeds present a characteristic morphology, nevertheless they are highly polymorphic. Moreover, they are still identifiable after carbonization, mineralization or strong dehydration or waterlogging. The use of biometric ratios is a powerful approach within the taxonomic study of genus *Vitis* although the measurement of the seeds is often imprecise because of the fact that the stalk is often broken. Stummer (1911) proposed a morphometric index as the quotient seed breadth/seed length and described the domesticated grapevine seeds as bigger, longer, and with longer stalk. On the other hand, the wild grapevine pips were described as smaller, rounded, and with short stalks (Buxó, 1997; Valera *et al.*, 2023; Zohary and Hopf, 2000).

The identification of grapevine seeds using morphological characters has been under constant review (Levadoux, 1956; Terpó, 1976; Smith and Jones, 1990; Mangafa and Kotsakis 1996). In addition, in the last years linear discriminant analysis and elliptic descriptors method by Fourier Transform were added (Bouby *et al.*, 2013; Bouby *et al.*, 2021; Orrù *et al.*, 2012; Pagnoux *et al.*, 2014; Terral *et al.*, 2010,).

A great proportion of grapevine seed samples do not fit clearly into the categories defined by the Stummer's index and other formulas proposed to delimit the wild grapevine from cultivars (Smith and Jones, 1990; Mangafa and Kotsakis 1996; Valera *et al.*, 2023). This is due to the gradual nature of the transition between typically wild populations and domesticated cultivars. This gradation is associated both with the existence of hybrid swarms between wild and cultivated populations and with the different degree of transformation operated in cultivars, which makes some of them morphologically indistinguishable from the wild ones. In addition, introduced Amerindian and Asian species, occasionally became feral, their hybrids with *V. vinifera*, further complicating the situation.

We analyzed the morphology of grape seeds of various categories (cultivated, wild and feral) from Georgia and other regions with the following overarching aims:

1. To determine the possible existence of morphological pointers for introgression processes and the existence of hybrid swarms in wild and cultivated grapevine populations in Georgia.
2. To discriminate wild from feral individuals living both in natural habitats.
3. To detect those "primitive" cultivars which more closely resemble wild Georgian grapevines.
4. To assess in terms of probability the relationships of ancient Georgian grapevine seeds with wild or domesticated grapevine.

Material and Methods

Plant material

Thirty-five accessions of Georgian grapes were included in this research (Table 1): 15 accessions a priori represent *Vitis vinifera* Georgian autochthonous varieties and cultivars from various vinicultural regions of the Country; 15 are wild grapevine accessions belonging to *Vitis sylvestris*; 5 are feral from natural habitats but otherwise having ampelographic characters of cultivated *Vitis vinifera*. These seeds were analyzed within an ensemble of 782 *Vitis* seed samples at the Laboratory of Ethnobotany and Archaeobotany of the Murcia University (Spain) in order to have a wider framework (Supplementary Table 1).

The Georgian seeds have been collected from two field collections of Georgia. Both share the vinicultural region of Inner Kartli, they share similar climate and soil types and are ca. 64 km apart one from the other:

1: Jughaura collection (FAO code GEO036) of the LEPL Scientific – Research Center of Agriculture of Georgia and named after Academician S. Cholokashvili. The collection is located in Mtskheta district of Inner Kartli province of Eastern Georgia (Maghradze *et al.* 2022) and was established in 2008. The site receives 540–590 mm of average annual precipitation (Cola *et al.* 2017). The soils are meadow brown, and have good physical properties and the ability to retain water. The content of lime increases deeper in the soil (up to 18–20%); its pH is 7.8–8.1 and the humus content is 1.40–1.65%. It is poor in nitrogen and phosphorus and contains potassium in medium amounts. The planting layout is 2.3 m (between rows) × 1.3 m (between plants). All the grapevines are grafted on Kober 5 BB (*Vitis berlandieri* × *Vi-*

Table 1: List of Vitis accessions from Georgia.

Code	Genus	species	Accession Name	Usage	Berry color	Province of Origin	Type of accession
721	*Vitis*	*vinifera*	Asuretuli Shavi*	w/t	B	Lower Kartli	Cultivar
713	*Vitis*	*vinifera*	Budeshuri Tetri	w	W	Kartli	Cultivar
708	*Vitis*	*vinifera*	Buza	w	B	Kartli	Cultivar
710	*Vitis*	*vinifera*	Chinuri	w	W	Kartli	Cultivar
717	*Vitis*	*vinifera*	Chitistvala Meskhuri	w	W	Samtskhe-Javakheti	Cultivar
707	*Vitis*	*vinifera*	Gorula	w/t	W	Kartli	Cultivar
718	*Vitis*	*vinifera*	Kartlis Tita	t	W	Kartli	Cultivar
712	*Vitis*	*vinifera*	Kharistvala Meskhuri	t	W	Samtskhe-Javakheti	Cultivar
720	*Vitis*	*vinifera*	Kishuri	t	W	Kartli	Cultivar
709	*Vitis*	*vinifera*	Kisi	w	W	Kakheti	Cultivar
714	*Vitis*	*vinifera*	Orbeluri Ojaleshi	w	B	Racha-Lechkhumi	Cultivar
719	*Vitis*	*vinifera*	Otskhanuri Sapere	w	B	Imereti	Cultivar
711	*Vitis*	*vinifera*	Meskhuri Mtsvane	w	W	Samtskhe-Javakheti	Cultivar
716	*Vitis*	*vinifera*	Rkatsiteli	w	W	Kakheti	Cultivar
715	*Vitis*	*vinifera*	Saperavi	w	B	Kakheti	Cultivar
698	*Vitis*	*sylvestris*	Samebis seri 08	w	B	Kakheti	Wild
696	*Vitis*	*sylvestris*	Meneso 01	w	B	Inner Kartli	Wild
694	*Vitis*	*sylvestris*	Nakhiduri 11	w	B	Lower Kartli	Wild
695	*Vitis*	*sylvestris*	Sabue 03	w	B	Kakheti	Wild
699	*Vitis*	*sylvestris*	Kvetari 04	w	B	Kakheti	Wild
702	*Vitis*	*sylvestris*	Nakhiduri 15	w	B	Lower Kartli	Wild
704	*Vitis*	*sylvestris*	Ninotsminda 01	w	B	Kakheti	Wild
701	*Vitis*	*sylvestris*	Chkhumi 04	w	B	Racha-Lechkhumi	Wild
706	*Vitis*	*sylvestris*	Skra 01	w	B	Inner Kartli	Wild
703	*Vitis*	*sylvestris*	Delisi 06	w	B	Inner Kartli	Wild
700	*Vitis*	*sylvestris*	Mokhva	w	B	Imereti	Wild
695	*Vitis*	*sylvestris*	Tedotsminda 15	w	B	Kartli	Wild
705	*Vitis*	*sylvestris*	Barisakhos gadasakhvevi	w	B	Inner Kartli	Wild
693	*Vitis*	*sylvestris*	Tedotsminda 25	w	B	Innet Kartli	Wild
692	*Vitis*	*sylvestris*	Ninotsminda 02	w	B	Kakheti	Wild
691	*Vitis*	*vinifera*	Dighomi 01	t	W	Innet Kartli	Feral
690	*Vitis*	*vinifera*	Naghomari 01	w	B	Racha-Lechkhumi	Feral
687	*Vitis*	*vinifera*	Ninotsminda 09	w	B	Kakheti	Feral
689	*Vitis*	*vinifera*	Tedotsminda 01	w	B	Inner Kartli	Feral
688	*Vitis*	*vinifera*	Tedotsminda 22(2)	T	W	Inner Kartli	Feral

Codes and abbreviations: *Female variety. Usage of grapes for: w (wine), t (table), w/t (wine/table = means double uptitude grapes). Berry color as B (black, red-blue, rose), W (White and other non-colored)

tis riparia) rootstocks. The rows' direction is North to South. The pruning system is Double-Guyot (20–24 buds/vine). The *Vitis vinifera* subsp. *sylvestris* collection site (Latitude 41.90, Longitude 44.76, 513 m a.s.l.) contains 75 accessions of wild and feral grapes, collected in different natural habitats from several of Georgia's regions (Fig. 1), propagated and planted here since 2014.

2: The Skra Germplasm repository (FAO code GEO015). Collection located in village Skra, Gori District, Inner Karli province of Eastern Georgia., 640 m a.s.l. The collection was established in 2008, in the Georgian viticulture and winemaking region of Shida Kartli by the Institute of Horticulture, Viticulture and Oenology. The distance between rows is 2.5 m and the distance between grapevines is 1.5 m. All the grapevines are grafted on Kober 5 BB (*Vitis berlandieri* × *Vitis riparia*) rootstocks. The rows' direction is North to South. The scheme of pruning is double Guyot system with 12-16 winter buds/vine.

Accessions were collected in different locations in the provinces of Georgia (Table 1, Fig. 1)

Fig. 1: Provinces of Georgia from where grapevine accessions were analyzed.
Note: The provinces’ name are marked with a green rectangle light green filled in the case of being collected accessions from there. Image: D. Maghradze.

Characters studied

Each seed was individually described according to 20 characters (Fig. 2). Eleven are quantitative: total length, breadth and thickness of the seed, breadth of the beak at the junction with the body and at the seed base, length of the beak in dorsal and in ventral view, thickness of beak at the junction, length and breadth of the chalaza scutellum, and distance from the chalaza apex to the seed apex (Rivera *et al.* 2007). Six are allometric: width/length index, width/thickness index, prism volume index, beak length/seed length index, beak width/beak length index, and chalaza width/length index.

The qualitative characters are three: Contour type (assessment of shape), with five states (ovoid, quadrangular, triangular, rounded and pentagonal), arrangement of the fossettes, with four states (parallel, furcate, convergent and divergent) and presence/absence of radial furrows.

Quantitative and qualitative characters were measured and analyzed using digital scaled images. 10 seeds of each sample were individually placed, except when the number of seeds available was inferior, on a plasticine support with a built-in scale to be photographed in dorsal, ventral and lateral view with the camera of the Samsung A40 device and measured using the open-source Fiji software (Schindelin *et al.*, 2012). All photographs were taken under the same zoom conditions. Also, scale images of fossilized and archaeological seeds from specialized literature have been used for measurements. The characters were recorded in an Excel spreadsheet where the allometric relationships were automatically calculated using algorithms.

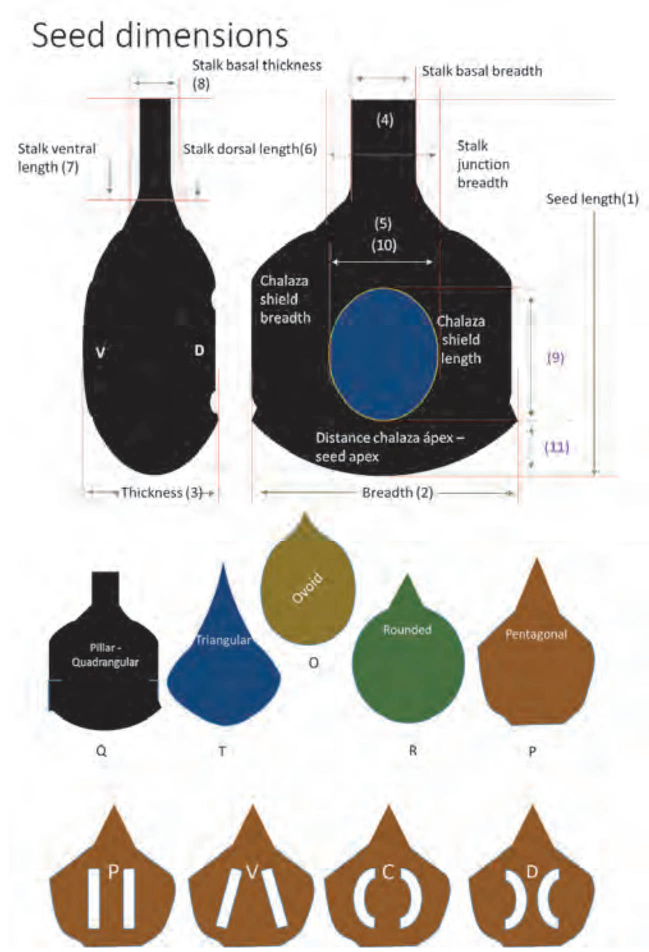

Fig. 2: Seed morphological characters.
Abbreviations: P: parallel; V: furcate; C: convergent; D: divergent. Image: D. Rivera.

Morphometric indexes

Stummer's Index

Stummer (1911) proposed an index based on the allometric relationship between seed width and seed length. This index allows differentiating quite effectively the extreme forms, but intermediate values are found in both wild and cultivated populations (Table 2). Stummer's index values ranging from 0.44 to 0.53 would be exclusive to cultivars, while 0.76 to 0.83 would be unique to Austrian wild/ferals. Values between 0.53 and 0.76 were found in both cultivars and wild vines. Levadoux (1956) has shown that this index has limited validity and is not useful for distinguishing wild from cultivated vines.

Facsar-Perret's index

Perret in 1997 proposed a new index based on the allometric relationship between the length of the beak or column and the total length of the seed. Apparently, this index allows to differentiate quite effectively between wild and cultivated populations, with the border situated between 18 and 19 (Table 3). This index was previously proposed by Facsar (1970), Terpó (1976) and Facsar and Jerem (1985), with slight discrepancies with the threshold values.

Mangafa and Kotsaki's indexes

The formulae proposed by Mangafa and Kotsakis in 1996 were successfully applied to local Greek samples of, both, modern seeds, and archaeological remains. The four formulae (Table 4) are based on the combined use of relationships and constants involving variables such as seed length (L), stalk length (LS), and chalaza position (PCH).

Domestication index

Since the above indices serve the same purpose, to separate wild from domesticated forms, but their results differ from case to case, the combined use of the six indices may provide a better ability to discriminate seeds from wild from cultivated grapevines (Obón *et al.* 2024; Valera *et al.*, 2024). The combined domestication or the wild index are calculated individually for each seed using [1], where *NIT* means indexes exceeding, above or below, the threshold value and *NI* means indexes considered:

$$\theta = \sum_{i=1}^{n} NIT_i / \sum_{i=1}^{n} NI_i \qquad [1]$$

Θ = WI. Threshold values for recognizing a seed as wild: Stummer >75, Perret <19, Mangafa and Kotsakis F1 <-0.2, Mangafa and Kotsakis F2 <-0.2, Mangafa and Kotsakis F3 <0 and Mangafa and Kotsakis F4 <-0.9. The sum of the wild index and the domestication index values, which is complementary to the previous one, will always be equal to one.

Θ = DI. Threshold values for recognizing a seed as domesticated are as follows: Stummer ≤75, Perret ≥19, Mangafa and Kotsakis F1 ≥-0.2, Mangafa and Kotsakis F2 ≥-0.2, Mangafa and Kotsakis F3 ≥0 and Mangafa and Kotsakis F4 ≥-0.9.

Possible values of the DI, domestication index, range from 0 to 1, with intermediate values, in the present case based on six indices these are: 0.17, 0.33, 0.5, 0.67, and 0.83. Seeds with index values between 0.67 and 1, both included, would undoubtedly be domesticated seeds, and those with values between 0 and 0.33 would be wild. In the present work the value 0.5 is tentatively interpreted as wild.

We usually work with samples consisting of several seeds, which in the case of modern populations, wild or cultivated, usually come from the same cluster, although not always. There are three relevant parameters when inferring from the results of individual seeds the characteristics of the whole sample:

- The mean μ (WI), of the wild combined index values for individual seeds, ranging from 0 to 1. Similarly, is calculated μ (DI).
- The standard deviation σ (WI) of the WI index values. Similarly, is calculated σ (DI).
- The proportion of seeds within each sample exceeding the threshold, PW, proportion wild, ranging from 0 to 1.

Other useful parameters are: The Wild Sum/2 (WS/2) for each sample, which is the mean value of μ (WI) and PW; the minimum value of the DI, min (DI) and ρ (DI), the range of DI values (Rivera *et al.* in press.).

Hybridization index

The standard deviation of the DI index values has shown to be useful to distinguish hybrids and hybrid swarms from pure wild and pure domesticated populations, thus the choice of an alternative name for this as HI (Hybridization Index). Values above 0.2 of the standard deviation of DI index points to the hybrid or mixed nature of the sample (Rivera *et al.* in press).

Table 2: Stummer's index for wild and domesticated grapevine seeds[1].

Range of values	Taxa
44-53	*V. vinifera*
54-75	Intermediate or hybrids
76-83	*V. sylvestris*

[1] Formula: B/L × 100.

Table 3: Facsar – Perret's index for wild and domesticated grapevine seeds[1].

Range of values	Taxa
12-18	*V. sylvestris*
19-30	*V. vinifera*

[1] Formula: (LS/L) × 100. LS: stalk lengt, L: seed length.

Table 4: Mangafa and Kotsakis's indexes for wild and domesticated grapevine seeds [1].

Range of values	Taxonomic information
Range of values (Formula 1)	Seed classification
< -0.2	Wild grapes
-0.2 < × < 0.2	Wild grapes (64.7% probability to be wild)
0.2 < × < 0.8	Domesticated grapes (76.2% probability to be cultivated)
> 0.8	Domesticated grapes
Range of values (Formula 2)	Seed classification
< -0.2	Wild grapes
-0.2 < × < 0.4	Wild grapes (64.7% probability to be wild)
0.4 < × < 0.9	Domesticated grapes (76.2% probability to be cultivated)
> 0.9	Domesticated grapes
Range of values (Formula 3)	Seed classification
< 0	Wild grapes
0 < × < 0.5	Wild grapes (90.1% probability to be wild)
0.5 < × < 0.9	Domesticated grapes (63.3% probability to be cultivated)
> 0.9	Domesticated grapes
Range of values (Formula 4)	Seed classification
< -0.9	Wild grapes
-0.9 < × < 0.2	Wild grapes (90.1% probability to be wild)
0.2 < × < 1.4	Domesticated grapes (63.3% probability to be cultivated)
> 1.4	Domesticated grapes

[1] Formula 1:-0.3801 + (- 30.2 LS/L) + 0.4564 PCH– 1.386 L + 2.88 PCH/L + 9.4239 LS)
Formula 2: 0.2951 + (-12.64 PCH/L – 1.6416 L + 4.5131 PCH + 9.63 LS/L)
Formula 3:-7.491 + (1.7715 PCH + 0.49 PCH/L + 9.56 LS/L)
Formula 4: 0.7509 + (-1.5748 L + 5.297 PCH – 14.47 PCH/L)
LS, stalk length; L, seed length; PCH, chalaza position.

Multivariate analysis

Variables

The data matrix consists of 782 samples (rows) and 227 columns of variables resulting of the segmentation in mutually excluding states or classes of the 20 primary variables described above, in the form of a spectrum of frequencies expressed in percentages with the following structure, from left to right: length (25 classes), width (21), thickness (9), width/length ratio (29), width/thickness ratio (10), volume (12), beak length in dorsal view (9) and in ventral view (9), beak length/seed length ratio (16), beak width at base (11), beak width (11) and beak thickness at junction with body (6), beak width/length ratio (9), chalaza shield length (18), chalaza shield width (6), chalaza width/chalaza length ratio (9), chalaza apex to seed apex distance (10), outline (5), dorsal radial grooves (2).

Data Analyses

The chi square dissimilarity index was calculated based on the above data matrix (Perrier *et al.*, 2003; Perrier and Jacquemoud-Collet, 2023). This measure expresses a value x_{ik} as its contribution to the sum x_i on all variables and is a comparison of unit profiles [2].

$$d_{ij}^2 = \sum_{k=1}^{K} \left(\frac{x_{ik}}{x_{i.}} - \frac{x_{jk}}{x_{j.}}\right)^2 \left(\frac{x_{..}}{x_{.k}}\right) \quad [2]$$

for *j≠i*.

Where d_{ij}: dissimilarity between units *i* and *j*; *i, j = 1, 2, ……, N* (samples, rows), N = 782; *k = 1, 2, ……, K* (variables, columns).

Where d_{ij} = 1 means varieties, *i* and *j* differ in all variables, and d_{ij} = 0 means varieties *i* and *j* are identical.

These pairwise dissimilarities can be represented in a multidimensional space, but in order to obtain meaningful graphic representation of these relationships in a two-dimensional plane, we used cluster analysis.

Cluster analysis is a term used to name a set of numerical techniques in which the main purpose is to divide the objects of study into discrete groups. These groups are based on the characteristics of the objects (Kovach, 2007). We used the minimum variance clustering (Ward's method) that focuses on determining how much variation is within each cluster. In this way, the clusters will tend to be as distinct as possible, since the criterion for clustering is to have the least amount of variation (Kovach, 2007). Ward's method produces a single tree. For the graphic representation, we opted for the software Figtree version 1.4.4. (Rambaut, 2018).

The use of distance-based trees to allocate archaeological seed samples is not new. Pagnoux *et al.* (2015) assigned archaeological grape seeds to the groups defined by UPGMA cluster analysis; the tree is based on Mahalanobis distances among comparison grapevine wild individuals and cultivars. Rivera *et al.* (2014) tentatively allocated archaeological *Phoenix* seed samples using a method based on the Ward's tree.

Allocation of samples to categories and taxa

Bayes-Laplace theorem

For the interpretation of unknown-origin seed samples, we adopted a Bayesian approach. We try to answer the question: What is the conditional probability that a seed or seed sample of unknown adscription belongs to a determined *Vitis* taxon Θ_i given that it presents the domestication index value x_j and/or it belongs to the cluster y_j? The framework is based on the knowledge provided by hundreds of comparison samples (ca. 600) whose taxonomic identity we "a priori" know in each case not only from the morphology of the seeds but also from the study of the grapevine plant from which the sample was collected. This allows us to construct a discrete joint probability function $p(X,\Theta)$ that assigns a posterior probability value to each particular combination of a *Vitis* taxon and a domestication index value or of a *Vitis* taxon and a Ward's tree cluster.

The Bayes' rule [1] allows to approximate the answer.

$$p(\theta|x) = \ p(x|\theta)p(\theta)/p(x) \qquad [1]$$

Where $p(\Theta|x)$ is the posterior probability distribution for the parameter Θ given a single observed value of the variable $X=x_j$, in our case the degree of domestication, which is represented by the domestication index value, which ranges from 0 (clearly wild) to 1 (cultivar with fully domesticated traits).

When considering the Bayes' rule in terms of individual probabilities formula [1] can be read as [2]

$$posterior\ probability = \frac{likelihood\ x\ prior\ probability}{marginal\ likelihood} \qquad [2]$$

Given a value for the data, for instance $X=x_4$ and a specific value for the parameter Θ (*Vitis* taxa), such as, $\Theta=\vartheta_3$, we get [3]

$$p(\theta_3\,|\,x_4) = p(x_4\,|\,\theta_3)p(\theta_3)/p(x_4) \qquad [3]$$

In [3], both likelihood $p(x_4\,|\,\theta_3)$ and marginal likelihood $p(x_4)$ are values that can be calculated on the basis of the joint distribution generated from the comparison samples. The prior probability $p(\theta_3)$ can also be calculated as the sum of probabilities of this taxon given the distribution of all *x* values, also on the sample data alone. But the very nature of the prior allows the inclusion of data on regional prevalence of the different taxa from other well-established sources of evidence. In this study we have paid attention in the case of domesticated grapevines to the geographical variation in the proportions of the different *Vitis vinifera* "*proles*" and, in the case of the rest to the ratio *V. sylvestris*/*V. caucasica*. Further we pay attention to the low relevance of the fossil vines, introduced as an outgroup, and treated as such.

Application to the Georgian seeds question

The identification process in all cases is based on using a frame of comparison that is based on the availability of an extensive set of seed samples previously identified not only by the morphology of their seeds but by a whole set of taxonomic characters obtained from the analysis of the plants as a whole. In a Bayesian concept, the elaboration of this framework and the selection of the set of samples is part of our prior knowledge. And it generates "a priori" probabilities, related to the joint distribution of the parameters and the sampled variables, which constrict our results. Following within the Bayesian approach, we can advance in our evaluation of the probabilities of the different mutually exclusive and exhaustive hypotheses by considering all the available "a priori" evidence on the relative frequencies of the hypotheses. Especially considering time and space constraints. For example, it is much less likely to find seeds of an American grapevine species in a European Neolithic site than that of *Vitis sylvestris* seeds. A careful elaboration of the "a priori" distribution of probabilities based on solid and logically coherent evidence is as fundamental as a clear definition of the different hypotheses and of the variable(s) to be considered.

The "a priori" or "prior" probabilities applied are based on the assumption that at least, 90% of the grapevines in the territory are cultivated and only 10% are wild. If we were to assume different proportions, the following probabilities would somewhat differ. Most of the old Georgian local varieties, according to the classification of Negrul (1946), belong to the Black Sea ecological-geographic group of varieties *Proles Pontica Subproles Georgica* Negr., and a small number belongs to the Oriental ecological-geographic group *Proles Orientalis Subproles Caspica* Negr. (Chkhartishvili 2008). However, based on the Georgian cultivars present in international repositories, notably The Institute for Grapevine Breeding Geilweilerhof, Germany, and those described in the *Ampelographia SSSR* (Baranov *et al.* 1946; Frolov-Bagreev *et al.* 1953-1956) we detected a higher proportion of *Proles Orientalis* in the fraction as follows:

- Oriental cultivars. *Proles Orientalis* Negrul, divided into *Subproles Antasiatica*, 0.18 and *Subproles Caspica*, 0.30.
- Western and Mediterranean cultivars in a broad sense. *Proles Pontica* Negrul, 0.33 and *Proles Occidentalis* Negrul, 0.07.
- Varieties with intermediate characteristics resulting from hybridization between the previous groups, 0.02.
- *Vitis sylvestris*, we include the variability inherent to western wild grapevines that do not descend from cultivated plants, 0.002.
- Feral grapevines, which are descended from cultivated plants and although they show partial reversion to ancestral characters, they conserve traits derived from domestication, 0.01.
- Wild grapevines from the Caucasus or other eastern regions, their probability is small, but we do not rule them out. They are divided into direct hybrids of wild Caucasian grapevines with cultivars 0.03; purely Caucasian feral varieties, 0.02 or wild Caucasian grapevines, 0.03.

- American grapevine species, we should not rule out that an American vine could be present in the Caucasus in form of escaped or feral individuals or populations, 0.01.
- Eastern Asian grapevine species, they are unlikely but given the ancient connection facilitated by the Silk Road it is not impossible their presence even in recent times, 0.01.
- Finally, fossils that are extremely unlikely, but we do not rule out the survival of a living fossil, 0.00001.

In the Bayesian method, we can sequentially combine the evidence resulting from the study of several variables by concatenating results in which the posterior probability distribution of the first analysis will be used as the a priori distribution for the second and so on (Rivera *et al.* 2020). In the present study, we combine the results of the morphometric indices summarized in the domestication index for each of the seeds as a starting analysis and then use the results of the multivariate analysis in terms of the assignment of each of the samples to one or another of the 13 clusters.

Results

The morphological differences between the seeds of wild grapevines from the Caucasus and those from Western Europe are negligible, except that the former are slightly more voluminous as a result of greater length, width and thickness, but to a lesser degree (Table 5). The mean value of the domestication index, on the other hand, is significantly higher in the former, 0.41, compared to 0.29. Consequently, the wild index is higher in wild Western European populations (0.71) than in Caucasian populations (0.53), inversely the domestication index is lower, 0.29 and 0.43 respectively.

The above allows the following reading: wild Caucasian populations show a higher degree of introgression with domesticated populations, hence the higher average domestication value, which seems to confirm what we see below:

Among the Caucasian wild samples there is more heterogeneity both within and between samples, leading to higher hybridization index values μ (HI) = 0.19, compared with μ (HI) = 0.12 in the Western European wild grapevines (cf. Rivera *et al.* in press).

One thing we must not forget is the presence in the European and Caucasus wild populations of American grapevine species and their interspecific hybrids and those hybrids with *Vitis vinifera*, which have been used as rootstocks since the end of the 19th century (Levadoux 1956, Laguna 2003, 2004 and 2006). The seeds of American grapevine species tend to show a greater similarity to those of *Vitis sylvestris*, so that introgressions with European wild grapevines would lead to a reinforcement in the parameters analyzed of the wild traits.

The maximum value reached by the hybridization index is around 0.5. This index presents in feral grapevines the average value of 0.07 in the Caucasus and 0.11 in Western Europe, which would suggest that in both cases the feral grapevines are basically cultivars growing in natural habitats. In natural hybrids (wild × cultivar) the hybridization index values μ (HI) = 0.26 (maximum 43) for Caucasian populations and μ (HI) = 0.21 (maximum 34) for Western European populations.

The multivariate analysis considers the variability contributed by the set of characters analyzed (Fig. 2), including also those considered in the various indices. In this way we obtain a seed classification (Fig. S1) that can be compared with that obtained from the indices.

The samples from Georgia were allocated to clusters 3, 4, 5, 8, and 9 in the Ward's minimum variance tree (Fig. S1). Clusters 3 to 5 include predominantly cultivars while clusters 8 and 9 do contain wild grapevines.

The combined analysis of the domestication index values and cluster assignment through the joint distribution of those values with the different previously identified comparison samples allows us to approximate the identification of the Georgian samples to be verified, as previously indicated using the Bayesian conditional probability approach considering the alternative research hypotheses. Some examples of the different seed types can be found in Fig. 3. The results are presented in Supplementary Table 2 and summarized in Fig. 4.

Concerning archaeobotanical materials we had access to the analysis of images from one Georgian sample: Grape pips from Shulaveri Gora (Georgia) c. 6000 BC (Tbilisi Archaeological Museum) (Maghradze *et al.* 2020) that we compared with those from Bronze Age levels of Norabak site in the area

Table 5: Morphological characterization of *Vitis sylvestris* seeds from South Caucasus and Western Europe.

		L	B	T	Stummer's Index	LCH	BCH	WI	DI
South Caucasus (60 seeds/6 SI)	**Maximum**	6.1	4.7	3.8	1.0	1.6	1.5	1.00	1.00
	Mean	**4.9**	**3.8**	**2.7**	**0.8**	**1.0**	**0.9**	**0.53**	**0.47**
	Minimum	3.7	3.1	2.0	0.6	0.6	0.6	0.00	0.00
		L	**B**	**T**	**Stummer's Index**	**LCH**	**BCH**	**WI**	**DI**
Western Europe (180 seeds/28 SI)	**Maximum**	5.9	4.5	3.8	1.0	1.8	1.7	1.00	1.00
	Mean	**4.8**	**3.6**	**2.7**	**0.8**	**1.1**	**0.9**	**0.71**	**0.29**
	Minimum	3.4	2.5	1.8	0.6	0.5	0.3	0.00	0.00

Abbreviations: L: length (mm), B: breadth (mm), T: thickness (mm), LCH: chalaza shield length (mm), BCH: chalaza shield breadth (mm), WI: "wild" index, DI: domestication index, SI: Samples.

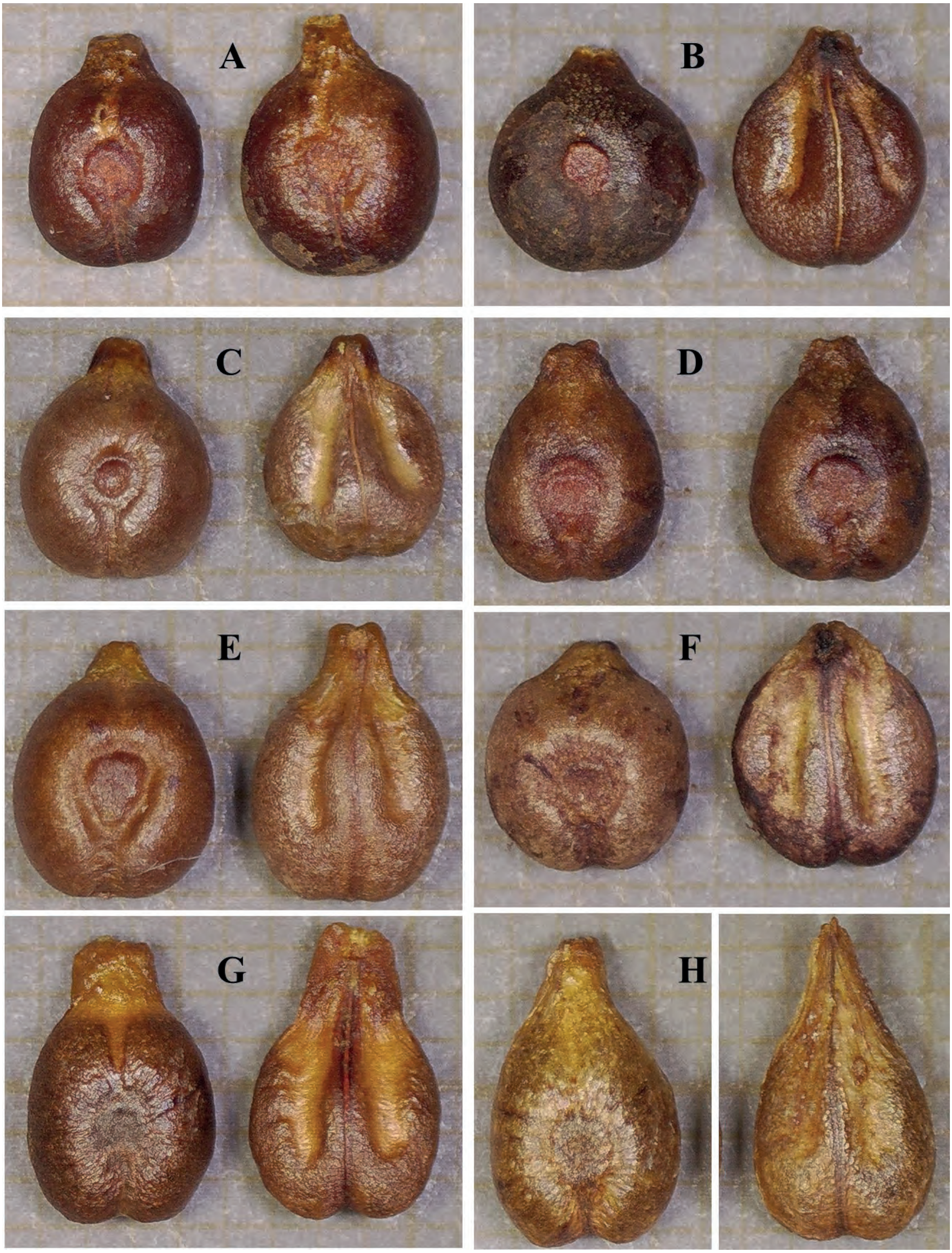

Fig. 3: Selected grapevines seed samples wild and cultivated from Georgia.

Digital Microscopy Images, Background: 1 millimeter orange graph paper. Samples and DI values of the ensemble of the sample: A, 687 Ninotsminda DI= 0.33-1; B 689 Tedotsminda DI= 0.33-1; C, 698 Samebis Seri DI= 0.33-0.67; D, Kvetari; E, 700 Mokhva DI=0.33-1; F, 706 Skra DI= 0.17-0.83; G, 710 Chinuri DI=1; H, 716 Rkatsiteli DI=1. Further information in Table 6.

of Lake Sevan (Armenia) (Hovsepyan, 2017) and Neolithic Aratashen and Aknashen sites in Armenia (Hovsepyan and Willcox 2008). While those from Shulaveri Gora presented a domestication index between 0.67 and 1 and a relatively high probability of being feral or hybrid, what is surprising, the seeds from Neolithic Aratashen area, despite their domestication rate being 0.67, slightly above the threshold, presented high probabilities of being wild (0.58) or hybrid (0.30) with *V. sylvestris* or even with an Asian species, this is consistent with the identification made by Hovsepyan and Willcox (2008). Although the Sulaveri sample is allocated close (Fig. S1) to one determined as feral from La Algaida de San Lucar (Spain) by Rivera *et al.* (in press), it is part of cluster 3 where we also find modern cultivars from Georgia. The seed from Norabak Bronze Age Site (Hovsepyan, 2017), presents a DI value of 1, suggesting a fully domesticated cultivar.

Discussion

Categories within Georgian wild and cultivated grapevine resulting of seeds analysis

During our study, in most cases, the probability values obtained for each individual seed allowed them to be clearly assigned to one of the alternative groups, with a predominance of more or less pure wild grapevines and those of the *Proles Pontica* cultivars. However, as can be seen in Supplementary Table 2, there were frequent cases suggesting a more or less direct hybrid origin of the sample.

Table 6 summarizes the heterogeneity detected within samples and the coincidence or not with the a priori classification of accessions. Nine among the 15 cultivars are reallocated as such. However, the remaining six show heterogeneity suggesting primitiveness or hybridization. Overall feral accessions show a similar pattern of heterogeneity suggesting more hybridization or primitiveness than mere escaped cultivars. In the case of the fifteen samples of wild accessions, a high intra-sample heterogeneity has been detected, which may be an intrinsic feature of Caucasian wild grapevines, but may also be due to various levels of introgression with cultivated landraces. Overall our results confirm the observations made by Ramishvili (1988) on the Georgina populations of wild grapevine. It is worth mentioning the Kvetari 04 sample which shows traits that bring it closer to wild populations from Western Europe and wild Asian and American species.

This is complicated by the fact that in some samples the heterogeneity detected was so high that it assigned some seeds to clearly wild groups, others to hybrids or to domesticated ones, which would confirm the hybrid or mixed character of the individual sample (Fig. 4 samples denoted with stars).

This led us to recognize further a categorization system based on probabilities and domestication index as follows.

Domesticated cultivars:

1. Domesticated grapevines likely cultivars of *Proles Pontica* (*Pontica* probability 0.58-0.89) (DI (Domestication index) = 1).
2. Domesticated grapevines likely resulting from modern cultivars of *Proles Pontica* introgressed with *Proles Orientalis* and *Occidentalis* (*Pontica* 0.52-0.75, *Caspica* 0.09-0.25, *Occidentalis* 0.11-0.12) (DI = 0.67-0.83).
3. Domesticated grapevines likely modern cultivars of *Proles Orientalis* hybridized with *Proles Pontica* (*Pontica* 0.35, *Antasiatica* 0.27, *Caspica* 0.29). (DI = 0.93-1).

Hybrid grapevines:

1. Hybrid grapevines likely of *Proles Pontica* with wild Caucasian (Wild Caucasian probability 0.06, *Pontica* 0.37, Caucasian hybrid 0.26, Caucasian feral 0.1). (DI = 0.83).
2. Hybrid grapevines likely descendant from *Proles Pontica* cultivars (Wild Caucasian probability 0.03, *Pontica* 0.33, Caucasian hybrid 0.27, Other hybrids 0.1). (DI = 0.62-0.75).

Wild grapevines pure, introgressed or hybridized:

1. Wild hybrid grapevines likely introgressed with *Proles Pontica* domesticated cultivars (Wild Caucasian probability 0.25, Pontica 0.13, Caucasian hybrid 0.35, other hybrids 0.12) (DI = 0.5-0.55).

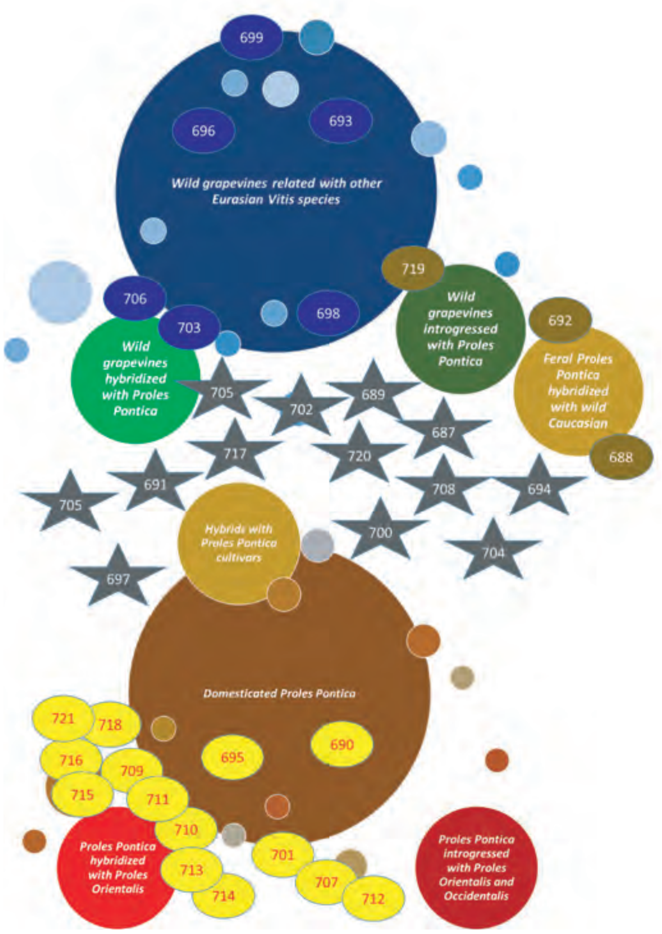

Fig. 4: Allocation of the different Georgian samples of wild and domesticated grapevines to the main groups.

Note: Represented with stars are samples that presented heterogeneous values in the different seeds analyzed within the sample enough to allocate with different probabilities these to the diverse hypothetic categories. Numbers are the three prime digits of the sample name as in Supplementary Table 2.

Table 6: Summary of the reallocation to groups of the analyzed samples.

Code	Accession Name	Usage	Berry color	Province of Origin	Type of accession	Groups	DI range
			Pure domesticated cultivars				
709	Kisi	w	W	Kakheti	**Cultivar**	PO, CA, AN	1
710	Chinuri	w	W	Kartli	**Cultivar**	PO, CA, AN	1
711	Meskhuri Mtsvane	w	W	Samtskhe-Javakheti	**Cultivar**	PO, CA, AN	1
713	Budeshuri Tetri	w	W	Kartli	**Cultivar**	PO, CA, AN	1
714	Orbeluri Ojaleshi	w	B	Racha-Lechkhumi	**Cultivar**	PO, CA, AN	1
715	Saperavi	w	B	Kakheti	**Cultivar**	PO, CA, AN	1
716	Rkatsiteli	w	W	Kakheti	**Cultivar**	PO, CA, AN	1
718	Kartlis Tita	t	W	Kartli	**Cultivar**	PO, CA, AN	1
721	Asuretuli Shavi*	w/t	B	Lower Kartli	**Cultivar**	PO, CA, AN	1
			Cultivars with seed heterogeneity suggesting introgression with wild individuals or persistence of ancestral traits				
717	Chitistvala Meskhuri	w	W	Samtskhe-Javakheti	**Cultivar**	CV, PO, CH	0.33-1
719	Otskhanuri Sapere	w	B	Imereti	**Cultivar**	PO, CH (HY, CV)	0.5-0.83
708	Buza	w	B	Kartli	**Cultivar**	PO, CH (CH, CV, HY)	0.5-1
720	Kishuri	t	W	Kartli	**Cultivar**	PO, CH, CV	0.5-1
707	Gorula	w/t	W	Kartli	**Cultivar**	PO, CA, AN, OC	0.67-1
712	Kharistvala Meskhuri	t	W	Samtskhe-Javakheti	**Cultivar**	PO, CA, AN, OC	0.83-1
			Wild with seed morphology corresponding to domesticated cultivars				
701	Chkhumi 04	w	B	Racha-Lechkhumi	**Wild**	PO, CA, AN, OC	0.83-1
695	Sabue 03	w	B	Kakheti	**Wild**	PO, CH, CF, HY	0.67-1
694	Nakhiduri 11	w	B	Lower Kartli	**Wild**	PO, CH	0.67-1
			Feral with seed heterogeneity suggesting hybridization wild – cultivars				
688	Tedotsminda 22(2)	T	W	Inner Kartli	**Feral**	PO, CH, CV, HY	0.33-0.83
687	Ninotsminda 09	w	B	Kakheti	**Feral**	PO, CV, CH	0.33-1
689	Tedotsminda 01	w	B	Inner Kartli	**Feral**	PO, CH, CV, HY, AM, ASI	0.33-1
690	Naghomari 01	w	B	Racha-Lechkhumi	**Feral**	PO, CS, OC, AN	0.67-1
691	Dighomi 01	t	W	Innet Kartli	**Feral**	PO, CH, CV	0.5-1
			Wild accessions with seed heterogeneity suggesting hybridization wild – cultivars				
692	Ninotsminda 02	w	B	Kakheti	**Wild**	PO, CH, HY, CV	0.33-0.83
702	Nakhiduri 15	w	B	Lower Kartli	**Wild**	PO, CH (CF, CV; HY)	0.5-1
704	Ninotsminda 01	w	B	Kakheti	**Wild**	PO, CV, CH	0.5-1
705	Barisakhos gadasakhvevi	w	B	Inner Kartli	**Wild**	PO, CV, CH	0.5-1
			Wild accessions with seed morphology typically will but with slight heterogeneity suggesting hybridization wild – cultivars				
695	Tedotsminda 15	w	B	Kartli	**Wild**	CV, CH, PO	0.67-0.83
703	Delisi 06	w	B	Inner Kartli	**Wild**	CV, PO, CH	0.5-0.83
706	Skra 01	w	B	Inner Kartli	**Wild**	CV (PO, CH)	0.17-0.83
693	Tedotsminda 25	w	B	Innet Kartli	**Wild**	CV (CH, PO)	0.17-0.67
698	Samebis seri 08	w	B	Kakheti	**Wild**	CV (CH, PO)	0.33-0.67
696	Meneso 01	w	B	Inner Kartli	**Wild**	CV, CH, PO	0.33-1
700	Mokhva	w	B	Imereti	**Wild**	CV (PO, CH)	0.33-1
			Wild accession with peculiar seed morphology suggesting primitiveness and relationships with Asian species				
699	Kvetari 04	w	B	Kakheti	**Wild**	CV (AS	0-0.83

Abbreviations: AM: American species, AN: *Proles Orientalis Subproles Antasiatica* Negrul, AS: Asian species, CA: *Proles Orientalis Subproles Caspica*, CH: Caucasian hybrids, CV: Wild Caucasian, HY: Hybrids, OC: *Proles Occidentalis* Negrul, PO: *Proles Pontica* Negrul.

2. Wild grapevines likely hybridized with *Proles Pontica* cultivars (Wild Caucasian probability 0.28-0.44, *Pontica* 0.32-0.46, Caucasian hybrid 0.19-0.23). (DI = 0.63-0.88).
3. Wild grapevines likely hybridized with other Eurasian or American species (Wild Caucasian 0.3, Caucasian hybrids 0.33, Other hybrids 0.13, American 0.13, Asian 0.11). (DI = 0.33).
4. Wild grapevines of the Caucasus likely related to Asian *Vitis* species (Wild Caucasian probability 0.17, Asian 0.64) (DI = 0).
5. Wild grapevines of the Caucasus (Wild Caucasian probability 0.52-0.85) (DI = 0.17-0.83).

Georgian wild grapevines

Vitis sylvestris as described by Gmelin (1806) is a central European wild grapevine, apart from the prelinnaean synonyms linked to Switzerland, Gmelin lists several localities of West Germany and East France around Karlsruhe and Strasbourg. The diversity of *Vitis sylvestris* is very high due to geographical isolation of ancient populations of Tertiary origin, but also to introgression with local cultivars. For Caucasian and west Asian wild grapevine, Negrul, proposes that *Proles Occidentalis* originated from the wild form *aberrans*, with glabrous or hairy, dissected leaves which is still found growing in the forests of Kuba District in Azerbaijan (Sosnovszky, 1974) however this is not in agreement with the fact that *Proles Occidentalis* cultivars present a webby hairy cover and with the molecular evidence raised by Arroyo-Garcia *et al.* (2006) for an independent cradle in Western Europe.

The results of the morphometric study of wild grapevine in Georgia by Ekhvaia and Akhalkatsi (2010) reveal high morphological diversity. Morphological characters such as shape of leaf blade, number of lobes, pubescence type, coloration of internodes, leaves and berry skin, leaf vein lengths and angles between them and form of petiole sinus show high variability both within and among populations. The common characters for all studied populations were fully open tip of young shoot and distribution of tendrils on the shoot with an interval after two nodes and dioecy. These characters are considered as common for wild populations of *V. sylvestris*. Our results from the seeds analysis shows similar diversity that we attribute not only to intrinsic diversity but to the relevant impact of continued introgression. DNA analyses confirmed the proximity of wild, feral and cultivated grapevines in Georgia (Kikvadze *et al.,* 2023).

However, there are still many points that archaeobotany, and biomolecular genetics, have not been able to clarify, for instance the relevant difference in the spontaneous grapevines shown by Negrul's ampelographic research: from the *Vitis sylvestris*, autochthonous of Euro-Mediterranean and Pontic Europe, we get to its aberrans variety, which is spread in the Caspian area. Are the valuable traits of the latter merely the result of a natural selection during the last Ice Age (softer in the Caspian area than in the West)? Are such traits due to para-domestication or even to residuals of ancient domestications? (Forni 2012).

A study on plastid DNA sequence variation in wild grapevine from Azerbaijan, Georgia and Armenia revealed four different haplotypes. The AAA haplotype is restricted to eastern Georgia and Azerbaijan and is also found in cultivated grapevines, the ATA haplotype is randomly distributed throughout the study area and the ATT haplotype is distributed in the southern part of the study area, from the Black Sea to the Caspian Sea. The GTA haplotype was found in the southwestern part of Georgia (Musayev and Akparov, 2013; Musayev, 2014). Twenty-two genotypes of wild grapevine together with 139 autochthonous varieties were characterized by 20 SSR markers, demonstrating that wild accessions are well distinguished from the cultivated ones (Chkhartishvili and Maghradze, 2012).

The present study leads us to highlight the need to best identify and preserve as well as possible the true native wild populations of V. sylvestris (without introgressions), and to protect them with legislation, if necessary, because this work showed that their genetic modification by hybridization can be much higher than expected.

Hybrid grapevines

A high level of admixture was shown between Armenian and Georgian cultivars and a clear connection between *Proles Pontica* and *Proles Orientalis* (De Lorenzis *et al.*, 2015b). Which is consistent with the results of our Bayesian seed morphology analysis (Fig. 4). In the study of De Lorenzis *et al.* (2019) Georgian germplasm was grouped with the genotypes from the eastern Mediterranean Sea and South Italy but appeared as unique population with well-structured genotypes. The plastidial DNA study by Prazzoli (2017) showed a remarkable geographical pattern within the domesticated populations and also that the wild vines, regardless of their origin within Georgia, shared the haplotype with the domesticated populations of western Georgia.

Domesticated Georgian grapevines

The assumed model of domestication is relevant when interpreting the relationships between wild and cultivated populations of a given species. Four theories about the domestication process exist:

1. Monophyletic-monospecific where cultivated grapevine populations descend from wild local populations (Obón *et al.*, 2007).
2. Monophyletic-bispecific theory proposes that cultivated populations come from an extinct ancestor which could also be the ancestor of the wild grapevine, it supposes that cultivated grapevine and the wild one are different species (Sosnovszky, 1974).
3. Polyphyletic-multispecific theory defends that the local populations of the cultivated grapevine descend from independent wild ancestors. Therefore, each species of cultivated grapevine has a group of wild relatives.
4. Terpó (1978) proposed the hybridization between wild species of European grapevines and Central Asia as the origin of cultivated grapevine.

It is very likely that each of the above theories may encapsulate part of the complex evolutionary history of wild and cultivated grapevines in Eurasia.

De Lorenzis *et al.* (2015b) by genotyping of germplasm coming from Central Europe, Armenia, Azerbaijan, Georgia and Moldova by SSR markers identified 3 different groups, which was in agreement with the *Proles* classification proposed by Negrul (1946), by the genetic analysis using STRUCTURE and PCoA as follows:

1. Wine cultivars from the West (Central European Cultivars) = *Proles Occidentalis* (cultivars from Italian Peninsula, Central Europe and Iberian Peninsula)
2. Wine varieties from the East (Armenia, Georgia, Moldova) = *Proles Pontica* (varieties from Georgia to Balkans and the Anatolian Peninsula)
3. Table varieties from the East (Azerbaijan) = *Proles Orientalis* (Afghanistan, Armenia, Azerbaijan and Iran)

Over 1300 wild and cultivated grapevines collected around the Mediterranean basin and from Central Asia were tested with a set of 20 nuclear SSR markers by Riaz *et al.* (2018). Wild accessions from Georgia clustered with cultivated grape from the same area (*Proles Pontica*), but also with Western Europe (*Proles Occidentalis*). However, introgressions and hybridization occurring in wild populations with nearby domesticated grapevines should be further considered.

In the Vitis18kSNP assay (De Lorenzis *et al.* 2015a) cross hybridization events among native wild populations and cultivars are evidenced. Admixture proportions of wild and cultivated Georgian groups, as estimated by fast STRUCTURE, reflecting assignment probabilities of samples to each of the three groups, clearly show that domesticated G1 mainly hybridizes with domesticated G2. Furthermore, wild grapevine G3 presents a hybrid swarm mainly with G2, which is the best represented and incudes cultivars such as Rkatsiteli, Tavkeri and Zerdagi among others. This largely confirms the previous nuclear microsatellite-based results of Imazio *et al.* (2013).

Prazzoli (2017) using a set of 21 microsatellites, comprising the standard set of markers for genetic identification, analyzed plant material from the Caucasus. The phylogenetic analysis was performed by comparing accessions of *V. vinifera*, including *Proles Pontica*, *Subproles Antasiatica*, *Subproles Caspica*, *Proles Occidentalis*, Georgian accessions (wild and domesticated), as well as European *V. sylvestris*, and genotypes from Armenia, Azerbaijan, Israel, Iran and Central Asia. In addition, rootstocks were selected as outgroup. The analysis confirmed that the Caucasian accessions belonged to the *V. vinifera* group. The Georgian clade was divided into two different clades, one comprising most of the Georgian cultivars and the other composed of a combination of wild and cultivated genotypes, showing similarity to that of European *V. sylvestris*. In addition, phylogenetic analysis showed a high rate of admixture between populations, particularly within the Central Asian accessions.

Our results from the analysis of the seeds confirm the relevance of *Proles Pontica* in Georgia and, despite the higher importance we *a priori* attributed to *Proles Orientalis* during the estimation of our "initial belief" or "prior knowledge" that represents what we believe about a situation or parameter before considering new evidence or data, its weight is much lower, which coincides with the data of other authors. Volynkin (2008) based on 160 varieties belonging to the Georgian subgroup of the ecogeographical group of the Black Sea Basin, *Proles Pontica Subproles Georgica* Negr., has shown that the mean sugar accumulation of the varieties under study varied from 14 to 24 g cm^{-3}, and that no correlation exists between the level of sugar accumulation and the length and the structure of the production period, and that all these parameters are variety-specific.

Reflections from our analysis on the conservation of genetic resources

History of conservation of Georgian grape genetic resources started since 1890, when the first grape collection was established in Sakara (Zestaphoni) of Western Georgia and after two years the first ampelography of Imeretian varieties was published by Vladimir Staroselskii (Staroselskii, 1893; Maghradze, 2008). The largest number of Georgian local varieties were collected during the 30s of the 20th century, by researchers working at the Institute of Viticulture and Winemaking of Georgia – among others was A.M. Negrul, working in that time in Telavi, where in the 1930s the first state-owned collection was planted to conserve 255 Georgian local varieties. Based on that materials several grape collections of different sizes were established in Georgia during the 20th century, notably in Dighomi with 3000 accessions, including 420 local cultivars and more than 30 wild and semi-wild forms.

Today there are eight different collections in Georgia. The State collection with 1000 accessions including local and introduced foreign varieties are located in Jighaura and belongs to the Scientific-Research Center of Agriculture. Others are private ones. A total of c. 1000 accessions is conserved, including local, introduced and breeded varieties, clones, and rootstocks, and wild and semi-wild forms of grapevine (only c. 30) (Maghradze 2008).

The information obtained from the analyzed samples in the present study that came from Jigharua and Skra repositories, given the high heterogeneity detected that we connect with hybridization, and suggests the convenience of a survey of the accessions is highly recommended. But in parallel our results would reflect a function as conservatory of domesticated grapevine diversity from the part of the riparian and other natural habitats where wild grapevine grows, and thus, the convenience of further analyzing this diversity from different viewpoints, widely sampling in the field and developing an extensive program of *in situ* and *ex situ* conservation.

Conclusions

Both wild grapevines and a good part of the cultivated grapevines analyzed from Georgia show a high heterogeneity in the morphology of their seeds, which seems to point to a very ancient history of domestication, interactions between wild and cultivated populations and preservation of ancestral traits. We must underline the extraordinary value that these hybrid and feral populations can have as a reservoir of grapevine genetic resources, especially descendants of ancient varieties possibly lost in cultivation at present. This is why the detailed ampelographic and genetic study of these populations is very necessary and urgent, as a further step in the development of innovations in the Georgian vineyard. Following the results obtained in this study, we recommend a greater effort in the consequent protection of wild grapevine populations, especially those with clearly native traits.

Supplementary Material

Supplementary Material can be found online at: https://doi.org/10.5073/vitis.2024.63.09

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Conflicts of interest

The authors declare that they do not have any conflicts of interest.

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