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Wild grapevine (*Vitis sylvestris* C.C.Gmel.) wines from the Southern Caucasus region

David Maghradze[1,2], Gagik Melyan[3], Vugar Salimov[4], Ramazi Chipashvili[5], Monserrat Íñiguez[6], Pilar Puras[6], Elena Melendez[6], Ramón Vaca[7], Carlos Ocete[8], Diego Rivera[9], Concepción Obón[12], Jose Manuel Valle[10], Alvaro Rodriguez-Miranda[10], Osvaldo Failla[11] and Rafael Ocete[8]

[1] Scientific – Research Center of Agriculture, Marshal Gelovani Ave. 6, 0159 Tbilisi, Georgia
[2] Faculty of Viticulture and Winemaking, Caucasus International University, Chargali Str. 73, 0141, Tbilisi, Georgia
[3] Armenian Academy of Viticulture and Wine-making, Pushkin str. 33/10, 375002, Yerevan, Armenia
[4] Institute of Viticulture and Winemaking, Mekhtiabad vil. Absheron distr. 0118, Baku, Azerbaijan
[5] Institute of Horticulture, Viticulture and Oenology, Agricultural University of Georgia, 0159, David Aghmashenebeli Alley 240, Tbilisi, Georgia
[6] Estación Enológica de Haro, Av. Bretón de los Herreros, 4. 26200. Haro, La Rioja, Spain
[7] Batle Winery, Palma de Mallorca, Camí de Coanegra, s/n, 07320 Santa Maria del Camí, Illes Balears, Spain
[8] University of Seville, San Fernando 4, 41004, Seville, Spain
[9] Departamento de Biología Vegetal. Facultad de Biología. Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain.
[10] Laboratorio de documentación geométrica del patrimonio. University of Basque Country *(UPV/EHU)*, Centro de Investigación Micaela Portilla, 01006 Vitoria – Gasteiz, Spain
[11] University of Milan, Via Celoria 2, 20133 Milan, Italy
[12] Departamento Biología Aplicada, EPSO, Universidad Miguel Hernández de Elche, 30312 Orihuela (Alicante), Spain

*Corresponding author: drivera@um.es

ABSTRACT

Grapevine domestication took place in the Caucasus area known as the Cradle of Viticulture, within or near the geographical area known as the Vavilov Triangle. The phytogenetic resources of *Vitis sylvestris* C.C.Gmel. have been previously collected and characterized, but the study on micro vinifications of wild grapevines from the Caucasus is new.

In the present document, seven grape samples from female individuals of wild grapevine growing in the South Caucasus region were investigated to assess their oenological profile.

Wine samples were obtained from the grapes collected from various populations of Armenia, Azerbaijan and Georgia in October 2013 and fermented by the native yeasts.

Parameters determined in the wines were, among others, the concentration of ethanol (3.63 % - 10.15 %), pH (3.30 - 4.20), total acidity (1.2 - 10.7 g/L of tartaric acid), total polyphenol index (1.81 - 89.8) and colour intensity (2.59 - 20.76). This wide range of values is due to the different environmental conditions, the level of ripeness of harvested grapes and their genetic diversity. These data were compared with those obtained in micro vinifications of wild grapevines in Western Europe and wines of several international cultivars.

The results of our research demonstrated, that the must of wild grape could be used to improve traditional wines giving them more colouration.

KEYWORDS

micro vinification, river-bank forests, ethnobotany, *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi, wine, Caucasus

INTRODUCTION

The Eurasian wild grapevine, *Vitis sylvestris* C.C. Gmel., constitute the dioecious parental of *Vitis vinifera* L. cultivars, which are usually hermaphrodites (Rivera and Walker, 1989; This *et al.*, 2006; Zohary, 2000). The Eurasian wild grapevine has received very diverse taxonomic treatments, from the rank of variety to one of the species. This implies the use of the subsequent valid names, depending on the accepted level: *Vitis vinifera* var. *sylvestris* Willd., *V. vinifera* subsp. *sylvestris* (Willd.) Hegi or *V. vinifera* subsp. *sylvestris* (C.C. Gmel.) Hegi, and *V. sylvestris* C.C. Gmel. (Ferrer-Gallego, 2019).

Fossils of this autochthonous vine for Eurasia appear within sediments dated from the end of the *Pliocene* (Sémah and Renault-Miskovsky, 2004). At present, these wild populations are disseminated in natural ecosystems from the Iberian Peninsula to Hindu Kush (Arnold *et al.*, 2002). Some populations of this liana can be also found in the African Maghreb (Ocete *et al.*, 2007). Their main habitats are river-bank forests, river mouths, flood plains, colluvial positions on the slopes of hills and mountains and coasts between the parallels 49º N (Rhine River, Germany) and 30º N (Ourika River, Morocco) (Iriarte *et al.*, 2013). In such places, soils are often renewed by flooding (Arnold, 2002; Maghradze *et al*., 2010).

Pallas (1799 - 1801), a German naturalist at the service of Empress Catherine II of Russia, reported the presence of countless wild grapevine populations in the Southern Caucasus. There were several individuals with large logs, some of them with the thickness of a ship's mast; their branches climbed on 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. Eyriés (1841) indicated that the grapevine grows in the gullies and plains of Southern Caucasus as in their primitive homeland. Thus, suggesting this area to be part of a centre of domestication for grapevine, which is consistent with recent molecular data: "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).

The Caucasus became even more relevant for understanding *Vitis sylvestris* diversity after the choice of a neotype for this taxon 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!).

The South Caucasus region is situated between the Black and Caspian seas, across several countries, notably Armenia, Azerbaijan and Georgia, and is an important refuge area for numerous plant species including sweet chestnut, walnut and wild grapevine (Aradhya *et al.,* 2017; Krebs, 2019; Ramishvili, 1988; Ramishvili, 2001). Several wild relatives of domesticated fruit species are present there in relic habitats in the Greater Caucasus mountain range (Huglin and Schneider, 1986; Vavilov, 1931). It constitutes the territory with the highest Eurasian grapevine diversity (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" (Figure 1) (Robinson *et al.*, 2013). The South Caucasus region has been postulated as the cradle of viticulture and winemaking (McGovern, 2003; 2004, McGovern *et al.*, 2017; Zohary, 2000).

In South Caucasus Region wild grapevine climbs on numerous tree and shrub species in open woodland (Ocete *et al.*, 2018). Uses of Caucasian wild grapevine include medicine; agriculture (pollination of female cultivars) and food (male flowers flavouring wines in Azerbaijan, and unripe fruits (verjuice) in marinades and special sauces (Maghradze *et al*., 2015b).

The Eurasian wild grapevine is considered a threatened plant genetic resource due to the overexploitation of riverine forests, and the establishment of orchards and public works. The importation of fungal diseases from North America, such as downy and powdery mildews strongly reduced natural populations (Ocete *et al.*, 2015). Furthermore, after *Phylloxera* infestation, there was a massive incorporation of North American *Vitis* species in Eurasian vineyards. They were used as root-stocks and in genetic improvement projects addressed at obtaining direct producer hybrids (French-American hybrids). Both kinds of plants showed a heavy invasive character as feral plants in wild habitats, highly competitive in the same habitats where lived autochthonous Eurasian wild grapevine (Ocete *et al*., 2007; Terpó, 1974).

Wild grapevine reproduces mainly by seed, differing from the established vegetative propagation of cultivars (Iriarte *et al.,* 2013; Revilla *et al*., 2010), and presents a higher level of genetic diversity, particularly in South Caucasian Region (Pipia *et al*., 2015). Genetic studies including haplotype distribution based on plastid DNA sequences show high levels of variation in wild grapevine (*V. vinifera* subsp. *sylvestris*) from the Greater Caucasus region (Pipia *et al*., 2012). In natural wild populations mutations affecting male vines can originate hermaphrodite individuals (Picq *et al.,* 2014). Early farmers selected hermaphrodite grapevines, presumably due to their higher production of grapes and easier cultivation, to establish the first vineyards outside river-bank forests (Forni, 2006, 2012; Scienza, 2004; This *et al*., 2006). However, some degree of dioecy coexisted in cultivation. The South Caucasus Region houses numerous female cultivars (97 out of 725 for the whole area, 53/414 in Georgia, 22/144 in Azerbaijan, and 22/171 in Armenia) (Negrul, 1970). In the years of intensive development of viticulture in Azerbaijan, it was carried out artificial pollination of functionally female grapevine varieties (Ag shany, Khatuni, Tavkveri, Nimrang and others) with pollen of male inflorescences of wild grapes to enhance the productivity of vineyards (Efendiyev, 1972).

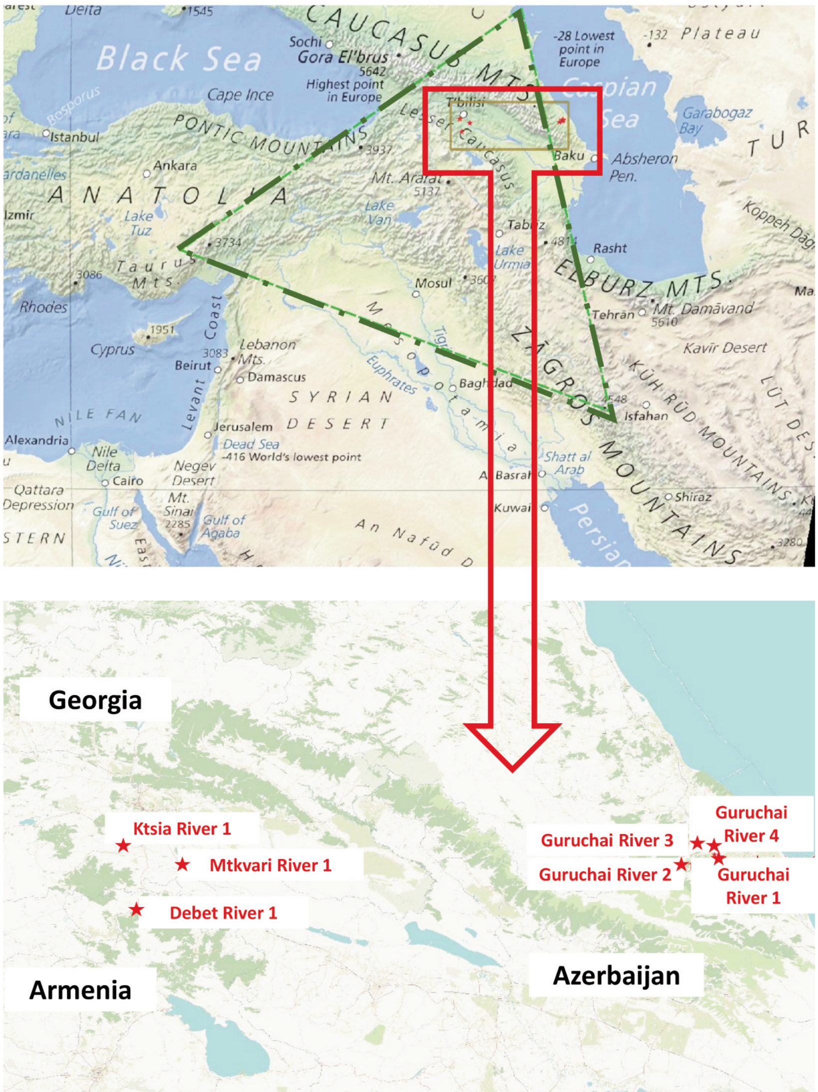

FIGURE 1. The “Vavilov’s Triangle” and sampled localities.

Shulaveri-Shomu culture existed on the territory of present-day Georgia, Azerbaijan and Armenia. The culture is dated to the 6th or early 5th millennia BC and is thought to be one of the earliest known Neolithic cultures. Some of the first wine production artefacts were found in the archaeological sites of Shulaveri Gora and Gadachrili Gora in South Georgia with other evidence of agricultural activities dated c. 8000 BP (Chilashvili, 2004; McGovern, 2003; McGovern *et al*., 2017) (Figure 2). Archaeological excavations in the Areni-1 cave complex in south-eastern Armenia revealed installations and artefacts dating to around 6000 BP that are strongly indicative of wine production (Barnard *et al.*, 2011).

It is necessary to remark that liquid products other than wine were obtained from grapes during the Prehistory and Antiquity. Grape must was used to improve ceramic pastes, at least, from the Bronze Age and grape vinegar was a very important food preserver used in beverages such as the "posca" consumed by Roman legions (Ocete *et al*., 2011c). The population of ancient Azerbaijan used wild grapes in food. Over time, local residents began to move wild grapevine closer to its homes and cultivate it. Remains of wild grapevine were found among the rocks of the ancient Gobustan and in the Khachmass region of Azerbaijan (Babayev, 1988).

The grapevine cross, or Saint Nino's cross, is a major symbol of the Christian Georgian Orthodox Church. Saint Nino of Cappadocia, who preached in Georgia in the 4th century AD, is represented as a girl holding up a cross made with shoots of grapevine tied using her own hair (Maghradze *et al.*, 2015a).

The Eurasian wild grape produces a rather astringent, small fruit with numerous seeds, hardly the kind of grape for making good wine. Its sugar content is relatively low and acids are high, as compared with the domesticated Eurasian cultivars, and the skin of its fruit is tough (McGovern, 2003). Therefore, it could be expected that wine obtained from these grapes would differ in certain analytic parameters from common wines.

An ampelography of selected native grape varieties of the six countries Azerbaijan, Armenia, Georgia, Moldova, Russia and Ukraine has been published. The identification, collection, characterization and conservation of the diversity of grapevine genetic resources was done 2004 - 2008 (Maghradze *et al.*, 2012).

FIGURE 2. Archaeological grape vine evidence.
A, Grape pips from ShulaveriGora (Georgia) c. 6000 BC (Tbilisi Archaeological Museum);
B, Large vessel with decorations imitating clusters of grapes supposedly used to have contained wine, c. 6000 BC (Tbilisi Archaeological Museum); Images: R. Ocete.

According to the philosophy of the COST FA 1003 Action: "East-West Collaboration for Grapevine Diversity and Exploration and Mobilization of Adaptive Traits for Breeding" (2010 - 2014) an expedition to collect and conserve plant genetic resources of grapevines from the South Caucasian Region was carried out in 2013.

Georgian cultivated and wild grapevine has been described (Chkhartishvili and Maghradze, 2012; Ocete *et al.*, 2012) and genetically characterized (De Lorenzis *et al.*, 2015; Ekhvaia *et al.,* 2014; Imazio *et al.*, 2013;Imazio *et al.*, 2013), but not the winemaking with wild grapevine of this country, likely in Azerbaijan (Salimov and Musayev, 2012) and Armenia (Melyan and Gasparyan, 2012).

We believe it is important to make it clear that wild grapevines in the Caucasus are an important genetic resource for all the reasons above stated. The wild grapevines of the Caucasus have been studied and characterized genetically and morphologically but there is a lack of data of the characteristics of the wine they provide.

The wild grapes have been vinified since ancient times and are still used for this purpose both in

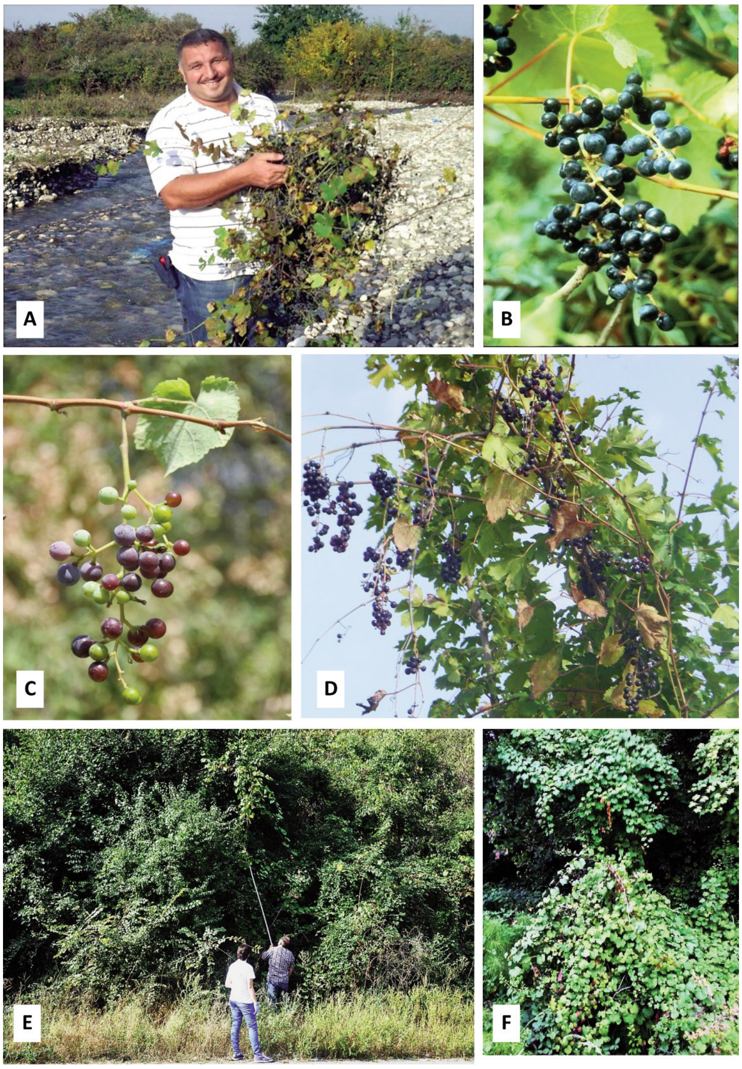

FIGURE 3. Harvest of wild grapes and habitats.
A, Harvest of wild grapes in Guruchai River (Azerbaijan); B, Ripe wild grapes, Guruchai River; C, Ripe wild grape from Ktsia River (Georgia); D, Fruiting wild grapevine in Guruchai River (Azerbaijan). E, Harvesting climbing grapevine (Georgia). F, Climbing wild grapevine and supporter (Azerbaijan). Images: D. Maghradze and V. Salimov.

TABLE 1. Geographic information of wild grape populations from their natural habitats in South Caucasus countries and characteristics of their grapes.

	Azerbaijan			
Place/ Population	Guruchai River 1	Guruchai River 2	Guruchai River 3	Guruchai River 4
Latitude	41°24'01"	41°26'03"	41°28'09"	41°27'43"
Longitude	48°26'37"	48°33'41"	48°33'59"	48°35'25"
Berry skin colour*	Blue-black	Dark red-violet	Blue-black	Blue-black
Berry shape*	Round	Round	Round	Round
Habitat	Remains of *Quercus iberica* forest in anthropized habitats	*Populus alba riparian* forest	Remains of *Quercus iberica* forest in anthropized habitats	Remains of *Quercus iberica* forest in anthropized habitats
Slope orientation, altitude and sun exposition**	Slightly north facing, 681 m a.s.l, sheltered	Slightly north facing, 407 m a.s.l, sheltered	Almost flat, 384 m a.s.l, sun-exposed	Almost flat, 346 m a.s.l, sun-exposed
Average precipitation (mm)***	416	416	416	416

	Wine 5	Wine 6	Wine 7
	Georgia		Armenia
Place/ Population	Ktsia River 1	Mtkvari River 1	Debet River 1
Latitude	41°29'22"	41°22' 43"	41°07'16"
Longitude	44°40'51"	45°03' 25"	44°45'16"
Berry skin colour*	Dark red-violet	Dark red-violet	Dark red-violet
Berry shape*	Round	Round	Round
Habitat	*Punica granatum* and *Crataegus riparian* thicket	*Punica granatum* and *Elaeagnus riparian* thicket	Quercus iberica fores with *Fraxinus* and *Acer*
Slope orientation, altitude and sun exposition**	North facing, 421 m a. s.l., shaded	West facing, 277 m a. s. l., sun-exposed	Steep slope west facing, 652 m a. s. l., sun-exposed
Average precipitation (mm)***	495	370	650

* We follow the rules of IPGRI-UPOV-OIV (1997). ** Given that wild grapevine plants are climbing on different tree supports, the upper branches receive abundant light (Ocete *et al.*, 2018). ***Climate data from NOAA (2020): Guba for Guruchai River, Bolnisi for Ktsia River, Gardabani for Mtkvari River and Odzun for Debet River. Data on temperatures are not available.

the study area and in other places where wild grapevine grows (for example in Sardinia).

For all this, the aim of this work is: to characterize the wine that is obtained from wild grapes harvested in the several populations of Azerbaijan, Georgia and Armenia; to establish a preliminary characterization on the oenological potential of wild grapes within this geographical area; to know better the likely compositions of the wines produced before grapevine domestication.

MATERIALS AND METHODS

1. |Sampling

Harvest of grapes took place at the second middle of October 2013 in Armenia, Azerbaijan and Georgia in the wild grapevine populations free of the presence of feral cultivars and American root-stocks (Figure 3).

The coordinates of the different populations sampled along river-bank forests and flood plains are shown in Table 1 and Figure . These lianas climb on several species of the accompanying vegetation, such as *Carpinus betulus, Cornus mas*, *Corylus avellana*, *Crataegus caucasica, Mespilus germanica, Paliurus spina-christi, Prunus divaricata, Punica granatum*, *Cydonia oblonga*, *Pyrus caucasica*, *Quercus iberica, Salix capreae, Ulmus minor* among others (Ocete *et al.*, 2018).

All-female plants had red suborbicular berries, with diameter inferior to 1 cm. The skin of the grape is blue-black or dark red-violet (Table 1, Figure 3). The surface is covered with a thick wax layer.

2. Wine production and analysis

Bunches containing a considerable proportion of ripe grapes were selected among those available for harvest. High heterogeneity in the fruit set and ripening process observed in the same cluster (*millerandage*) is characteristic of wild grapevine populations (Trad *et al.*, 2017). The removal and separation of ripe grape berries from the stems (destemming) were done manually. Of each sample, 50 berries preselected as ripe were weighed to calculate what percentage is transformed into must. Only ripe berries were pressed using manual machinery. Given the small number of grapes available, only one sample from each locality (Table 1) was fermented, no replicas were made. The fermentation was carried out in the laboratory in glass jars, the first four weeks, and then transferred to bottles for transport, with the own yeasts that carried the berries (spontaneous fermentation), for a maximum of 15 days, with a fixed temperature of 20 ºC and daily stirring of the must with the skins of the berries. There was no addition of potassium metabisulfite. The samples were analyzed following the methods proposed by the OIV (2015) in a laboratory accredited under Quality Standard 17025 (ISO 2019), as follows:

- Ethanol: Near Infrared (NIR) (SpectraAlyzer WINE, ZEUTEC).
- pH and total acidity: Automatic potentiometry (Winelab Analyzer, FOODLAB-CDR, Florence, Italy - Tecnología Difusión Ibérica, Barcelona, Spain).
- Tartaric acid: Enzymatic (Cetlab 600, Microdom, Taverny, France - Tecnología Difusión Ibérica, Barcelona, Spain).
- Total polyphenol index: UV spectrometry (LAMBDA 265 PDA UV/Visible Spectrophotometer, cuvettes (1 mm pathlength), Perkin Elmer, Waltham, Massachusetts, USA).
- Colour intensity: UV-VIS spectrometry (LAMBDA 265 PDA UV/Visible Spectrophotometer, cuvettes (1 cm pathlength), Perkin Elmer, Waltham, Massachusetts, USA).
- L- Malic acid and volatile acidity: Enzymatic (Cetlab 600, Microdom, Taverny, France - Tecnología Difusión Ibérica, Barcelona, Spain).
- Reducing sugars: Autoanalyzer FCSA Q05 with Quaatro 39 (SEAL, Norderstedt, Germany - AXFLOW, Arsta, Sweden).

3. Comparison

To determine relationships within the wines obtained we calculated the pairwise differences between samples in form of a dissimilarity matrix.

The crude matrix consisted of 8 variables (ethanol content (% vol), total acidity (g/l), pH, tartaric acid (g/l), L-malic acid (g/l), colour intensity, total polyphenol index and reducing sugars (g/l)) and 18 units (defined using mean-sd, mean, and mean+sd values for each of the 6 samples). The matrix of chemical parameters was used to compute a dissimilarity matrix using DARwin V.6.0.17 (2018-04-25) (Perrier *et al.*, 2003; Perrier and Jacquemoud-Collet, 2006). The chi-square dissimilarity index was calculated. 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 [1].

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

where d_{ij}: dissimilarity between units i and j; x_{ik}, x_{jk}: values of variable k for units i and j; $x_{i.}$, $x_{j.}$: mean for units i and j; $x_{.k}$: mean for variable k; $x_{..}$: overall mean. K: number of variables.

To realistically represent individual relations, a hierarchical tree was constructed to describe the relationships between units (samples) based on the common agglomerative heuristic that proceeds by successive ascending agglomerations. For updating dissimilarity during the tree construction, the Ward criterion was adopted, which searches at each step for a local optimum to minimize the within-group or equivalently to maximize the between-group inertia. For the graphic representation, we have opted for the software Figtree version 1.4.3. (Rambaut, 2014). Analytical data of comparison samples were obtained from De Gianni (2015) (Nero d'Avola wine), Fogaça and Daudt (2012) (Brazilian *V. vinifera* cultivars), Revilla *et al.* (2016) (Spanish *V. vinifera* cultivars), Ocete *et al.* (2011b) (Spanish wild grapevine wines), Kang *et al.* (2008) (Traditional Korean wines are made by adding rice to grape juice and adding yeast), *V. rotundifolia* cultivars (Morris and Brady, 2004; Talcott, 2004).

RESULTS AND DISCUSSION

The must yield per kilogram of grapes harvested was situated between 16-17 %, due to the low proportion of pulp in the fruits. A wine volume of less than 250 ml was obtained in each of the micro fermentations, so the method of distillation with electronic densitometry was not applicable to calculate the ethanol concentration (v/v). Overall, the ethanol content measurement results were extremely low for a beverage that could be called wine (Table 2). This may be due to a low sugar content in the grapes.

Given that between the wine production in Georgia and the analysis in Spain, a period of several weeks elapsed (c. 40 days), it is likely that spontaneous malolactic fermentation occurred, which would explain why tartaric and malic acids represent only up to 50 % of total acidity.

The fact that the grapes have been fermented with local natural yeasts can influence the analytical characteristics of the experimental wines. Therefore, the differences between the wines are due not only to different origins and environmental conditions but to different yeasts as well.

Data on micro vinifications (Table 2) can be summarized as follows:

1. Azerbaijan

Wine 1 (Guruchai River 1). After fermentation, the percentage of ethanol recorded in this sample was 5.78%. This wine had good total acidity and showed a normal concentration of tartaric acid (Almela *et al*., 1996). The colour intensity was very low, similar to a rosé wine.

Wine 2 (Guruchai River 2). This wine showed a higher percentage of ethanol, 10.15 %. It is the maximum found in this region of the South Caucasus. Total acidity is adequate. The total polyphenol index is high, the colour intensity is good, 10.60 (it could be appropriate for a good quality red wine obtained from cultivars).

Wine 3 (Guruchai River 3). This wine showed a lower concentration of ethanol, 4.62 %. It has a low concentration of tartaric acid. The total polyphenol index could not be carried out due to the small volume of the sample.

Wine 4 (Guruchai River 4). This sample has a high total acidity, a low to normal amount of tartaric acid and only 5.04 % ethanol concentration. The intensity of the colour and the polyphenol index are normal according to its maturity level.

2. Georgia

Wine 5 (Ktsia River 1). The concentration of ethanol is 5.21 % vol. The intensity of colour and the polyphenol index present very good values. In this case, the phenolic maturity has been more in advance than the technological one as suggested by the sugars/acidity values ratio.

Wine 6 (Mtkvari River 1). The ethanol concentration is 7.2 % vol. The colour intensity and polyphenol index present decidedly acceptable values.

3. Armenia

Wine 7 (Debet River 1). The berries of this sample were so small, and with hardly any pulp, that barely any must volume was achieved and several determinations could not be completed. It showed the lowest percentage of alcohol of all microvinifications. Due to the few parameters determined (Table 2), it is not included in the comparison.

Considering all the results, analytical parameters mainly fall within the range of variation of cultivated grapevine wines. Ripeness level and sugar content are highly influenced by the degree of shade produced by botanical supporters (trees and shrubs) and the rest of the accompanying

TABLE 2. Wild grapes from South Caucasus countries: characteristics of their wines.

Parameters	Wine 1	Wine 2	Wine 3	Wine 4	Wine 5	Wine 6	Wine 7
	Values ($X \pm \sigma_x$)	Values ($X \pm \sigma_x$)	Values ($X \pm \sigma_x$)	Values ($X \pm \sigma_x$)	Values ($X \pm \sigma_x$)	Values ($X \pm \sigma_x$)	Values ($X \pm \sigma_x$)
Ethanol (%)	5.78 ± inap.	10.15 ± inap.	4.62 ± inap.	5.04 ± inap.	5.21 ± inap.	7.2 ± inap.	3.63 ± inap.
pH	3.58 ± 0.05	3.31 ± 0.05	5.64 ± 0.05	3.50 ± 0.05	3.30 ± 0.05	4.20 ± 0.05	-
Total acidity (g/L tartaric acid)	5.3 ± 0.4	7.7 ± 0.4	1.2 ± 0.4	10.7 ± 0.4	8.2 ± 0.4	6.1 ± 0.4	-
L-malic acid (g/L)	<0.10	<0.10	1.11 ± 0.22	<0.10 ± 0.22	0.90 ± 0.22	1.71 ± 0.22	<0.10
Tartaric acid (g/L)	2.30 ± 0.35	2.78 ± 0.35	0.59 ± 0.35	1.79 ± 0.35	3.24 ± 0.35	1.57 ± 0.35	
Reducing sugars (g/L)	1 ± 0.5	1.5 ± 0.5	1.7 ± 0.5	4.9 ± 0.5	1.3 ± 0.5	1.8 ± 0.5	-
Total polyphenol index	18.1 ± 0.9	**51.8 ±1.7**	-	29.9 ± 0.9	**56.50 ± 0.9**	**89.8 ± 0.9**	-
Colour intensity*	2.59 ± 0.058	**10.60 ± 0.058**	4.85 ± 0.058	3.76 ± 0.058	**20.19 ± 0.058**	**20.76 ± 0.058**	-

Notes: X: Average. σ_X: standard deviation. Inap., Inappreciable.
* For comparison with colour intensity of *Vitis vinifera* wines: cv Mencia (5.72 - 12.98 by Sudraud method and 16.43 - 17.21 by Glories method) and cv Alicante Bouschet (12.16 - 24.43 by Sudraud method and 13.73 - 28.08 by Glories method) from AOC Valdeorras, Galicia, NW Spain (Revilla *et al*., 2016); cv Merlot (between 4.3 - 11.0 by Glories method) from the Campahna Gáucha and Serra Gáucha regions of Brazil (Fogaça and Daudt, 2012). In bold samples of group A Figure 4.

vegetation in natural habitats, such as river-bank forests and flood plains (Ocete *et al*., 2018) (Figure 3). The concentration of anthocyanins of the skin of the berries that will form the pigmented polymers of red wines is also affected by shade and weather (Esteban *et al*., 2001; Fulcrand *et al*., 2006) and varies even in the same cultivar along different harvests (Revilla *et al*., 2009) and in wild grapevines (Benito, 2015; Revilla *et al*., 2010; Cantos *et al.*, 2017).

The ethanol percentage of normal samples varies between 4.62 % and 10.15 % (the abnormal sample 7 presented 3.63 %). The colour intensity varies between 2.59 and 20.76. It is necessary to remark that a wine is considered red, after the malolactic fermentation, when its intensity of the colour is 3.5 at least, for instance by the Regulatory Council of the Denomination of Origin Rioja (Spain) (Riojawine, 2019).

In general, ethanol levels and, sometimes, colour intensity values in Caucasus wines from wild grapevines are lower than those registered in micro vinification with wild grape samples from Sardinia (Italy) (Lovicu e*t al.,* 2009*)* and Andalusia, La Rioja, Castille and León and Navarre (Spain) (Ocete *et al*., 2011a; Ocete *et al*., 2011b). In the case of Spain, the maximum ethanol content was 14 %, registered in a sample harvested in Cáceres province (Extremadura) (Ayala *et al*., 2011) and the top colour intensity was 26.4 determined on a micro vinification from the Ega River (Álava province, Basque Country) (Meléndez *et al.*, 2015).

Concerning the classification, colour intensity and total polyphenol index determine three main groups (Figure 4) (cf. Table 2).

Group I is characterized by the highest values of total polyphenol index, 50 - 90 (mean 66) and colour intensity, 10 - 21 (mean 17.2). Total polyphenols and colour intensity are lower and similar for Groups II and II (17-31 for polyphenol index and 3-5 for colour intensity). Group II presents a lower tartaric acid content (0.2 - 0.9 g/L) in comparison with Group I (1.2 - 3.5 g/L) and Group III (1.4 - 2.7 g/L). Group II, also, presents an extremely low total acidity (0.8 - 1.6 g/L) and a higher pH (5.6 - 5.7). Finally, ethanol content was found not useful to recognize groups. Group I (Figure 4) include South Caucasian wild grapevine samples: two from Georgia and one from Azerbaijan. Whose compositions show similarities with some of the wild grapevine samples from Spain (Ayala *et al.,* 2011; Ocete *et al*., 2011a; Ocete *et al*., 2011b), Korean wines (Kang *et al*., 2008) and *Vitis rotundifolia* wines (Morris and Brady, 2004; Talcott, 2004).

Guruchai River samples 1, 3, 4, which form clusters II and III, produced wines that have shown similarities with those of *Vitis vinifera* cultivars and most wild Eurasian grapevine samples from Spain.

It is worth to highlight that, from a molecular marker perspective, South Caucasian populations belong to chlorotypes C and D, whereas

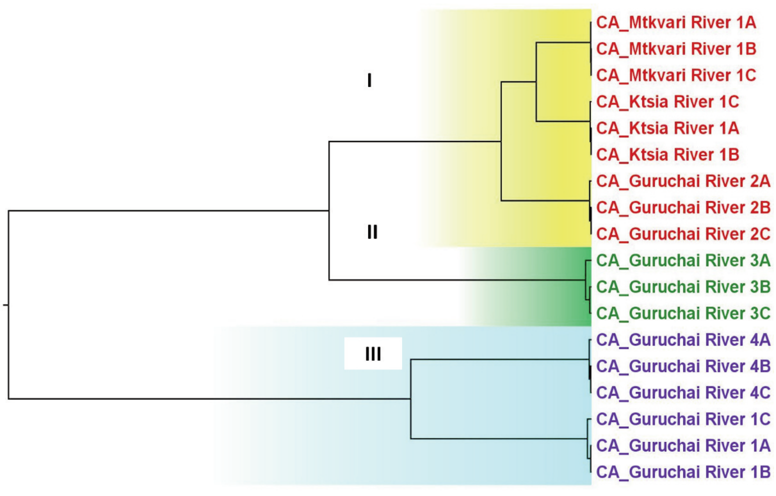

FIGURE 4. Relationships among Caucasus wine samples.
Note: Ward's minimum variance tree. A, B, C variants within each sample that were defined using mean-sd (A), mean (B), and mean+sd (C) values for each parameter).

Spanish ones belong to chlorotype A (Arroyo-García *et al*., 2006; De Andrés, *et al*., 2012).

All samples present reducing sugars not transformed in ethanol, at different concentrations. The high total polyphenol index and high acidity could be assumed responsible for the disruption of the normal action of yeasts. However, these are not significantly different from the levels in wines from cultivars. Moreover, Wine 3 has 1.7 g/L of reducing sugars and low polyphenols and acids content (Table 2). Therefore, we cannot associate this level of sugars with problems in fermentation due to the total polyphenol index and high acidity.

At the time of carrying out the analyses, the laboratory did not have the instruments for the study of aroma, so these data are not available, despite their interest. It would also be interesting to produce more wine to perform a sensory analysis. However, several points make it very difficult: the extremely low number of grapes produced by wild strains in their natural habitats of South Caucasus during episodes of drought, the inter-annual irregularity in the harvest and the difficult access to some of the populations.

The use of wild grapevine has been frequented for producing wine throughout history. Currently, the Eurasian wild grapevine is in the list of Endangered Plant Species of Georgia since 1982 (Chemonics, 2000). In Azerbaijan, people have always produced red and white wines. An interesting wine is the so-called "*gora sharab*", traditional of the region Guba-Khachmaz, Shaki-Zaqatala, Garabagh. For making this wine people use cultivated and wild grapes gathered in forest and riversides (Salimov and Musayev 2012).

In the Azerbaijan Research Institute of Viticulture and Wine-making buds and pollen of wild grapes are used as a flavour for preparing flavoured dessert wine like «nectar». This wine is characterized by a particular taste and unique flavour (Amanov, 2001; Amanov, 2005).

From unripe berries of wild grapevine, local habitants prepare healing juice, called «*gora suyu*» or «*gara suyu*». This juice is successfully used in the treatment of diabetes. Grapes contain numerous polyphenols, including the stilbene resveratrol, the flavanol quercetin, catechins, and anthocyanins that have shown potential for reducing hyperglycaemia, improving β-cell function, and protecting against β-cell loss. Therefore, with a low mean glycaemic index and glycaemic load, grapes or grape products may provide health benefits to type 2 diabetics (Rasines-Perea and Teissedre, 2017; Zunino, 2009).

An infusion of fresh leaves of the wild vine is widely used for the treatment of rheumatism (as a bath), as well as for improving eyesight (Damirov and Shukurov, 1985).

In Sardinia, a traditional wine is known as «*vinu de marxani*» or "*vino de volpe"* is made with the wild grape. Until the middle of the 20th century, shepherds of the mountainous area of Sulcis made their own wine with these wild grapevines, which they called *vino de caoprai* (Lovicu *et al*., 2009; Lovicu, 2013). Therefore, it has been traditional to make wine completely with wild grapes.

The potential contribution of wild grapes (wine 1, wine 2, wine 5) to lower the pH of the must by increasing the acid content, facilitating good wine conservation, is extremely limited by the considerable drop in alcohol that this addition can produce. Red wild grape wines (wine 2, wine 5 and wine 6), despite their high polyphenolic content that could help improve the preservation of base wines and add a higher concentration of anthocyanins, are of little use as improvers of wines made with cultivated varieties, for the same reason.

CONCLUSIONS

The wild grapevine populations cited in the present paper could be useful to make deeper oenological studies, such as the analysis of anthocyanin fingerprints. Wild grapevines with fruits rich in colour could be used to intensify the colour in red wines, as long as their low ethanol content can be resolved.

These wines (wines 2, 5 and 6) for their content in polyphenols could be used for improving the conservation of organic wines.

It is desirable that in these countries the traditional wine of the wild grapevine continues to be made and eventually added to the conventional local wines, which would confer certain original characteristics to the wines from the domesticated cultivars of the Eurasian grapevine.

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