WILD GRAPEVINE IN GEORGIA

Multidisciplinary Comparative Research to Unravel the Mystery of its Domestication

David Maghradze, Osvaldo Failla (Editors)

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This book represents an anthology of research and popularization works published in recent years on the theme of the wild grapevine (*Vitis vinifera* subsp *sylvestris* (C.C.Gmel.) Hegi in Georgia in the Southern Caucasus region. Moreover, it also included an original section dedicated to the ampelographic characterization of the accessions of Georgian wild grapevines recently collected in an *ex situ* collection. The anthological collection also included works not strictly dedicated to wild grapevine but considered fundamental for understanding the significance of the conservation and characterization of this species, an important component of wild flora and a precious genetic resource for viticulture not only in Georgia. The text is particularly addressed to the scientific communities in biology and viticulture, and experts involved in preservation of plant genetic resources.

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Preface

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David Maghradze and Osvaldo Failla (editors)

The wild grapevine *Vitis vinifera* L. subsp. *sylvestis* (C.C.Gmel.) Hegi is considered to be the supposed wild ancestral of cultivated grapevine *Vitis vinifera* L. subsp. *sativa* D.C. It is a typical representative of Georgian flora and it was widely spread almost on all territory of the Country in past. It is a part of the western Eurasian wild grapevine population and has significant importance as: i) founder for grapevine cultivation and breeding in the South Caucasus area 8.000 years ago and a probable key germplasm for investigation of the process of origin and evolution of the cultivated grapes in Europe; ii) endangered plant; iii) sources of genes for resistance or adaptation in the global climate change situation; iv) including for current viticultural research in the Word.

Investigation of the wild grapevine *V. sylvestis* has long history in Europe. It is going on actively during last decades with the purposes to survey its status, its linkage with domestication and evolution of European grapevine *V. vinifera* L. and origin of autochthonous germplasms (for example: Anzani et al. 1990, Failla et al. 1992, Campostrini et al. 1993, Olmo 1995, Arnold et al. 1998, 2002, Ocete et al. 1999, 2002, 2011, 2014, 2018, Grassi et al. 2002, 2003a, b, De Mattia et al. 2006, Di Vecchi et al. 2006, Schneidere et al. 2006, Arroyo-Garcia et al. 2006, 2016, Zecca et al. 2010, Ergul et al. 2011, Myles et al. 2011, Biagini et al. 2012, 2014, 2016, Bacilieri et al. 2013, Imazio et al. 2015, Arroio-Garcia and Revilla, 2013, Benito et al. 2016, Budić-Leto et al. 2018, Bonhomme et al. 2020, Zdunić et al. 2017, 2020, D'Onofrio, 2020, Cunha et al. 2020, Magris et al. 2021, Grassi & De Lorenzis, 2021). In this context investigation of wild grapevine in a such old agricultural country like Georgia from the South Caucasus is very significant.

The Law of Georgia on "Vine and Wine" (1998) underlines importance of wild grapevine and recognizes it as a "National Treasure" of the Country together with Georgian native varieties, being under protection of the State of Georgia. Based on this issue the state institutions have the responsibility to organize investigation of wild grapevine on the territory of Georgia, planting of discovered plants in a field collection for preservation and multidisciplinary research activities.

Research of wild grapevine *V. sylvestris* has been initiated in in the Caucasus included Georgia as well since the first part of the 19th century (Kolenati, 1846). The investigation became increasingly important after invasion of American fungal diseases and Phylloxera pest in the region since the second half of the 19th century (for example: Ruprecht, 1869; Sredinskii, 1874; DeCandol, 1885; Lipskii, 1885; Timofeev, 1892, Ballas, 1896).

The research and review of the data was carried out also later the 20th century (for example: Radde, 1901; Sosnovskii, 1925, 1946; Vavilov, 1931; Negrul, 1946; Burkach-Abramovich, 1953; M. Ramishvili, 1948, 1957,

1961; Grossgeim, 1962, Pruidze, 1966; Chamagua, 1968; Zhukovskii 1971, Cholokashvili, 1983; R. Ramishvili, 1970a,b, 1973, 1988, Red book, 1988). M. Ramishvili and R. Ramishvili collected 400 accessions of wildly growing grapevine from various regions of Georgia in Dighomi field collection of Tbilisi Agricultural Institute in 1967-1968. The wild grapevine was the main focus of research for R. Ramishvili (1988), who used to study this gene pool and even selected some prospective feral genotypes in Georgia in

cooperation with his students (Gozalishvili, 1985; Mandaria, 1987).

The new generation of the researchers (Ekhvaia and Akhalkatsi, 2010, Ekhvaia et al. 2014, Pipia et al. 2012) also advanced the knowledge about the different aspects of wild grapevine of Georgia.

Extensive investigation of wild grapevine of Georgia was renew since 2004 in the framework of the international project "Conservation and sustainable use of grapevine genetic resources in the Caucasus and Northern Black Sea area" leaded by the International Plant Genetic Resources Institute (IPGRI) and realized by the Institute of Horticulture, Viticulture and Oenology. Two following international projects GrapeGen06 and COST action FA1003, and one local project of National Wine Agency ("Research project on study of Georgian grape and wine culture") included also research of wild grapes of Georgia, as a part of the entire European gene pool, stimulated investigation of wild grapevine in the country. The collaboration with international universities and institutions became starting point for multi-disciplinary study of Georgian genetic resources including the wild grapevine also (for example, Maghradze et al. (2006, 2010, 2011), Ocete et al. (2012, 2018), Imazio et al. (2013), De Lorenzis et al. (2015a, b), Riaz et al. (2018) and others).

Focus of the local and international scientific societies on Georgian grape genetic resources increased interest to our wild grapevine also and Shota Rustaveli National Science Foundation issued to our working group the three-year grant "Wild grapevine of Georgia: research and preservation" (FR18-18474) in the category of Fundamental Research for the period of 2019-2022. The Scientific-Research Center of Georgia hosted implementation of this grant. The project was focused on research and conservation of wild grapevine including: monitoring of natural populations by expedition; establishment of a field collection for their conservation and further research; description of discovered plants by the GPS pointing and eco-botany methods; photo documentation; field evaluations of the wild populations and genotypes for ampelographic, phenological, viticultural and oenological traits; the results generated in this project will enable us to develop comprehensive long-term strategies for conservation and management of the wild ancestral species of grapevine. This publication also done in the framework of this Project.

Based on provided investigation and cooperation of the members of our research group with other institutions it became possible to make a number of publications, using wild grapes from Georgia, and mainly available in the peer reviewed journals and technical magazines. But we think that the compilation of research data in an anthological book would help the researches in providing information to the scientific community interesting about wild grapes. Based on this idea have been taken the decision about preparing of this publication.

The anthological collection also included works not strictly dedicated to wild grapevine but considered fundamental for understanding the significance of the conservation and characterization of this species, an important component of wild flora and a precious genetic resource for viticulture not only in Georgia.

The materials presented in this book cover wide aspects of wild grapes and are structured is the chapters like "Domestication", "Archaeobotany", "Study in Georgia", "Ecology and Conservation", "Genetic Diversity and Cultivar Linkage", "Genetics of Traits", "Pests and Diseases", "Must and Wines", "Microbiology", "Ampelography" and "Dissemination".

This book is a collaborative work of researches dedicated to analyze the recent status of Georgian wild grapevine investigation and arising some ideas for further development of study and preservation of this plant.

This book is particularly addressed to scientific communities in biology and viticulture, and experts involved in preservation of plant genetic resources; professors and students in the biological and agricultural fields.

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Chapter 1

Domestication

Early Neolithic wine of Georgia in the South Caucasus

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Chemical analyses of ancient organic compounds absorbed into the pottery fabrics from sites in Georgia in the South Caucasus region, dating to the early Neolithic period (ca. 6,000–5,000 BC), provide the earliest biomolecular archaeological evidence for grape wine and viniculture from the Near East, at ca. 6,000–5,800 BC. The chemical findings are corroborated by climatic and environmental reconstruction, together with archaeobotanical evidence, including grape pollen, starch, and epidermal remains associated with a jar of similar type and date. The very large-capacity jars, some of the earliest pottery made in the Near East, probably served as combination fermentation, aging, and serving vessels. They are the most numerous pottery type at many sites comprising the so-called "Shulaveri-Shomutepe Culture" of the Neolithic period, which extends into western Azerbaijan and northern Armenia. The discovery of early sixth millennium BC grape wine in this region is crucial to the later history of wine in Europe and the rest of the world.

Neolithic | wine | viticulture | Georgia | Near East

Following the last Ice Age, the Neolithic period in the Near East (ca. 10,000–4,500 BC) was a hotbed of experimentation, especially in the mountainous region extending west to east from the Taurus Mountains of southeastern Anatolia through the South Caucasus and northern Mesopotamia to the Zagros Mountains of northwestern Iran (e.g., refs. 1 and 2, including pertinent references). As the climate moderated and precipitation levels increased, especially between ca. 6,200–4,200 BC (*SI Appendix*), humans established year-round settlements. Permanent habitation allowed for a host of recently domesticated plants—including the "founder crops" of barley, einkorn wheat, emmer wheat, chickpea, pea, lentil, flax, and bitter vetch—to be efficiently raised, harvested, and stored. These developments were crucial in jump-starting the millennia-long upheaval and changes in human subsistence and culture known as the "Neolithic revolution" (3, 4).

Sedentary life, made possible by new, assured plant resources, was also accompanied by advances in the arts and crafts, such as architecture, weaving, dyeing, stone working, and woodworking. The invention of fired clay (pottery) containers sometime during the early seventh millennium BC (5, 6) had profound implications for processing, serving, and storing food and drink.

Human exploitation and cultivation of plants was not confined to staple cereals and legumes during the Neolithic. Fruits, nuts, tubers, herbs, and tree products are well-attested at Neolithic sites throughout the larger region. Among the fruit species, the wild Eurasian grape (*Vitis vinifera* sp. *sylvestris*) stands out, because its domestication as *V. vinifera* sp. *vinifera* became the basis of a widespread "wine culture" throughout the Near East and Egypt (1), which later spread to east Asia and across the Mediterranean to Europe (7–9), and then later to the New World. Today, there are some 8,000–10,000 domesticated cultivars of wine, raisin, and table grapes, with a range of colors from black to red to white. These cultivars owe their origins to human selection and accidental crosses or introgression between the incoming domesticated vine and native wild vines. These varieties account for 99.9% of the world's wine production and include famous Western European cultivars such as Cabernet Sauvignon, Sangiovese, Tempranillo, and Chardonnay (10).

The Near Eastern uplands have been described as the "world center" of the Eurasian grape (11), based on where the wild plant thrived and achieved its greatest genetic diversity. Indeed, DNA studies have shown that the wild vine of Anatolia is genetically closer to Western European cultivars than its wild counterpart there (12–16). Many cultivars in Georgia also have a close relationship to those in the West, including Pinot Noir, Nebbiolo, Syrah, and Chasselas (12).

Two important questions remain to be answered. Can more narrowly defined mountainous areas of greater Mesopotamia and the Fertile Crescent be delimited where the Eurasian grape first began to be made into wine and where it was subsequently domesticated? If so, when did these developments occur?

Archaeological Samples Chosen for Analysis

Our investigation, part of a larger Georgian project (17), sought to answer these questions by focusing on two archaeological sites

Significance

The earliest biomolecular archaeological and archaeobotanical evidence for grape wine and viniculture from the Near East, ca. 6,000–5,800 BC during the early Neolithic Period, was obtained by applying state-of-the-art archaeological, archaeobotanical, climatic, and chemical methods to newly excavated materials from two sites in Georgia in the South Caucasus. Wine is central to civilization as we know it in the West. As a medicine, social lubricant, mind-altering substance, and highly valued commodity, wine became the focus of religious cults, pharmacopoeias, cuisines, economies, and society in the ancient Near East. This wine culture subsequently spread around the globe. Viniculture illustrates human ingenuity in developing horticultural and winemaking techniques, such as domestication, propagation, selection of desirable traits, wine presses, suitable containers and closures, and so on.

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that were occupied during the earliest Pottery Neolithic period in Georgia, the so-called "Shulaveri-Shomutepe Culture" (SSC), dated to ca. 5,900–5,000 BC (18–20). The two sites are Shulaveris Gora, which gives its name to the period together with Shomutepe approximately 50 km downstream on the Kura River, and Gadachrili Gora (21). These sites are located within 2 km of one another in the province of Kvemo (Lower) Kartli, roughly 50 km south of the modern capital of Tbilisi (Fig. 1). Each is a small village, approximately 1 ha in area, of closely spaced mudbrick circular structures, 1–5 m in diameter, with interspersed pits and courtyards. The buildings are believed to be domestic residences, and the pits assumed to be for storage and/or refuse.

Fertile, rolling hills surround the sites on a high plateau at an altitude of >1,000 m ASL. Gadachrili Gora is presently bifurcated by the Shulaveris Ghele, a seasonal tributary of the

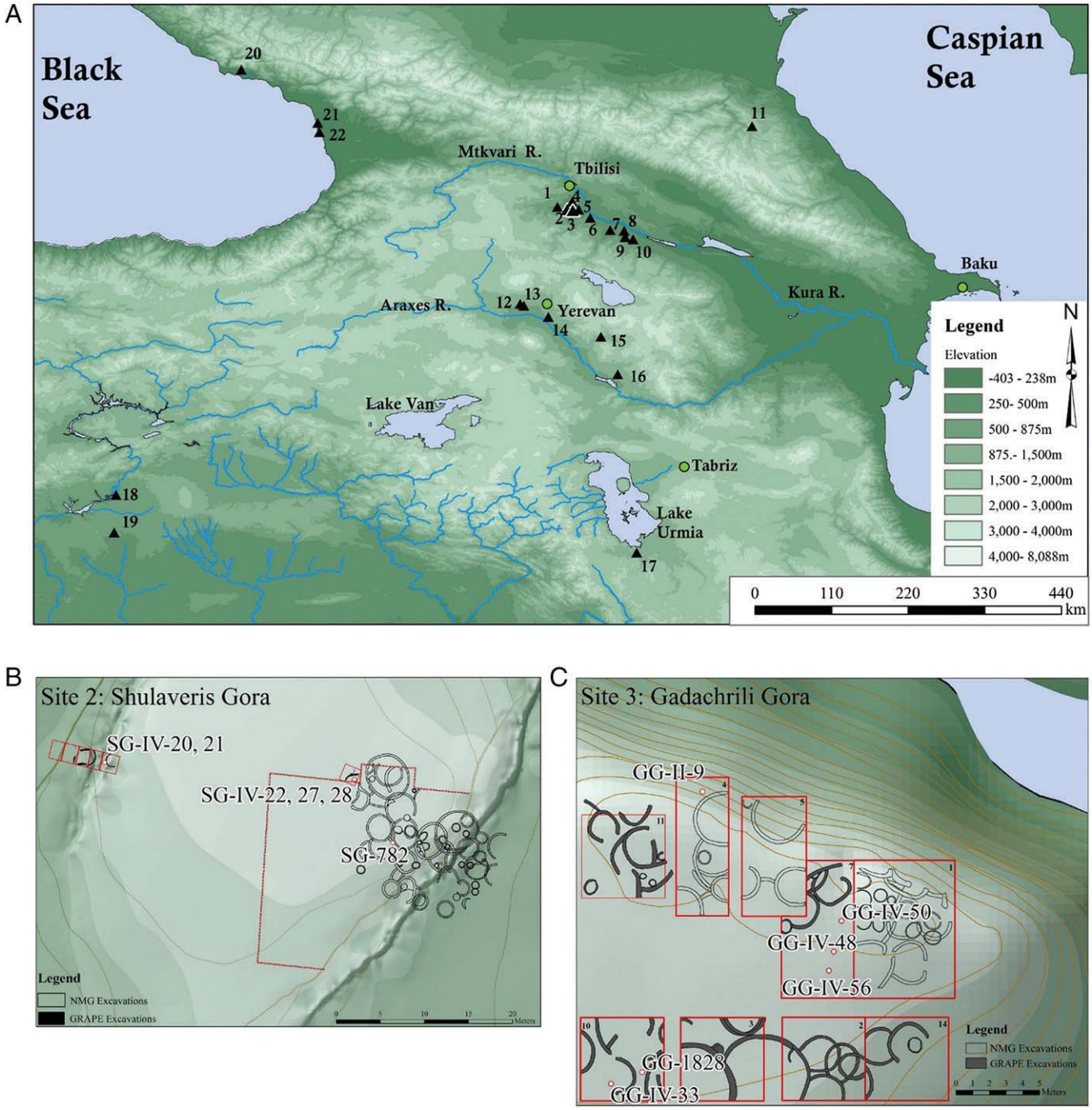

Fig. 1. Map of Shulaveri-Shomutepe Culture sites and other sites mentioned in the text (*A*) and the early Neolithic settlements of Shulaveris Gora (*B*) and Gadachrili Gora (*C*) showing the locations of the analyzed jar sherd samples that were positive for tartaric acid/tartrate. Site names: Arukhlo (1), Shulaveris Gora (2), Gadachrili Gora (3), Dangreuli Gora (4), Imeris Gora (5), Khramis Didi-Gora (6), Shomutepe (7), Haci Elamxali Tepe (8), Göytepe (9), Mentesh Tepe (10), Chokh (11), Aratashen (12), Aknashen (13), Masis Blur (14), Areni-1 (15), Kül Tepe (16), Hajji Firuz Tepe (17), Nevali Çori (18), Göbekli Tepe (19), Gudau River (20), Pichori (21), and Anaklia (22). GRAPE, Gadachrili Gora Regional Archaeological Project Expedition; NMG, National Museum of Georgia; R, river. Red lines indicate excavated areas and squares.

Khrami River that runs into the Kura, while Shulaveris Gora is roughly 0.5 km from the stream. The climate today is semiarid (steppe), with an annual rainfall of 350–550 mm and an average temperature of approximately 13 °C. Milder, better-watered conditions prevailed during the period ca. 5,900–5,000 BC (*SI Appendix*). The Eurasian grapevine was well adapted to the ancient climate and remains well adapted to the modern climate.

As is our standard practice in biomolecular archaeological investigations (22), we strove to obtain the best-dated, best-provenienced, and best-preserved samples possible. These criteria were met to a varying extent in this study. For example, we had previously analyzed two sherds (SG-16a and SG-782; Fig. 2 *B*–*C* and Table 1) from the 1960s excavations at Shulaveris Gora, which we designated as "borderline positives" for tartaric acid/tartrate (1), the principal biomarker of grape/wine in the Near East (*SI Appendix*), because of conflicting results from the less-sensitive chemical techniques that we used at that time. Moreover, the customary practice at that time was to "clean" sherds by washing them in dilute hydrochloric acid to remove calcium carbonate and other postburial accretions. In the process, ancient organics might well have been altered, even destroyed, to give "false positives." It was also later learned that the sherd with the highest apparent level of tartaric acid/tartrate (SG-16a) was collected from the surface of the site. Besides compromising the dating of this sherd, this also called into question the extent to which it had been subjected to environmental contamination and exposure to rain, which might have caused increased microbial activity and an elevated tartaric acid/tartrate content.

The opportunity to learn more and put the biomolecular archaeological investigation on a firmer, multidisciplinary foundation came when excavations at Gadachrili and Shulaveri were renewed in 2012–2013 and 2015–2016 (17). Many more radiocarbon dates from well-defined occupational contexts were obtained; coupled with advances in calibration curves and statistical evaluation, this has allowed for construction of a much tighter chronology for the early Neolithic than had been proposed in earlier publications (*SI Appendix*). Excavation and archaeobotanical techniques have also advanced since the 1960s, providing a finer-grained picture of how artifacts and ecofacts (i.e., plant and animal remains) were deposited and subjected to geological and chemical processes, as well as to human activity.

Fig. 2. (*A*) Representative early Neolithic jar from Khramis Didi-Gora (field no. XXI-60, building no. 63; depth, −5.45 to −6.25 m). (*B*) Jar base SG-16a, interior and cross-section. (*C*) Jar base SG-782, exterior. Note the textile impression on the base. (*D*) Jar base GG-IV-50, interior. (Photographs by Mindia Jalabadze and courtesy of the National Museum of Georgia.)

Table 1. Georgian early Neolithic pottery positive for tartaric acid by LC-MS-MS and their associated soil samples

Sample no.	Date (BC)	Provenience	Pottery type	Extract weight (mg)	Tartaric acid (ng/mg residue)*	Malic acid (ng/mg residue)*	Succinic acid (ng/mg residue)*	Citric acid (ng/mg residue)*
Gadachrili Gora								
GG-II-9, body sherd	ca. 5900-5750	Square BB-27, −2.73 m	Jar base sherd	NA	**134 ± 11***	**715 ± 86***	596 ±25*	**182 ± 3***
GG-II-9, soil	ca. 5900-5750	Square BB-27, −2.73 m	Associated soil	NA	20 ± 2*	491 ± 7*	630 ± 21*	10 ± 1*
GG-IV-33, disk base sherd	ca. 5700-5500	Square 10, Locus 4	Jar base sherd	1.2	**87 ± 6**	**998 ± 47**	**165 ± 13**	**186 ± 6**
GG-IV-62, soil	ca. 5700-5500	Square 10, Locus 4	Associated soil	0.8	7 ± 1	193 ± 33	32 ± 5	9 ± 0
GG-IV-50, pedestal base	ca. 5700-5500	Square 7, Locus 2	Jar base sherd	1.2	**17 ± 1**	**170 ± 13**	**31 ± 4**	**45 ± 1**
GG-IV-51, soil	ca. 5700-5500	Square 7, Locus 2	Associated soil	4.6	5 ± 0	91 ± 7	16 ± 0	5 ± 1
GG-IV-48, pedestal base	ca. 5700-5500	Square 7, Locus 2	Jar base sherd	4.3	**4 ± 1**	**50 ± 1**	**22 ± 1**	**6 ± 1**
GG-IV-54, soil	ca. 5700-5500	Square 7, Locus 2	Associated soil	4.5	1 ± 0	23 ± 1	7 ± 1	1 ± 0
GG-IV-56, flat base	ca. 5700-5500	Square 7, Locus 1	Jar base sherd	6.3	**39 ± 0**	**369 ± 22**	**54 ± 0**	**51 ± 0**
GG-IV-46, soil	ca. 5700-5500	Square 7, Locus 1	Associated soil	2.2	19 ± 0	312 ± 16	34 ± 4	20 ± 0
Shulaveris-Gora								
SG-16a, flat base	Early Neolithic	Surface	Jar body sherd	NA	**55 ± 1**†	**2028 ± 71**†	**198 ± 4**†	**58 ± 1**†
SG-782, pedestal base	ca. 5900-5750	Square BB, −0.8 m	Jar body sherd	NA	**8 ± 0**†	**387 ± 14**†	**56 ± 4**†	**15 ± 0**†
SG-IV-20, body sherd	ca. 5900-5750	Square 2, Locus 2	Jar base sherd	6.1	**4 ± 0**	**97 ± 2**	**12 ± 1**	**34 ± 0**
SG-IV-21, soil	ca. 5700-5500	Square 2, Locus 2	Associated soil	7.1	3 ± 0	56 ± 0	12 ± 1	5 ± 1
SG-IV-22, soil	ca. 5700-5500	Soil, Neolithic levels	Site soil	9.6	2 ± 0	17 ± 2	3 ± 0	1 ± 0
SG-IV-27, soil	ca. 5700-5500	Soil, Neolithic levels	Site soil	8.8	2 ± 0	18 ± 1	4 ± 0	1 ± 0
SG-IV-28, soil	ca. 5700-5500	Soil, Neolithic levels	Site soil	14.6	1 ± 1	9 ± 1	4 ± 1	1 ± 0

Numbers in bold highlight concentrations for ancient sherds that are higher than their corresponding soils. NA, not applicable.
*Except for the GG-II-9 samples, which are reported as nanograms of organic acid per gram of sherd/soil material (ng/g or ppb), all concentrations are cited as ng/mg (ppm) of extracted residue.
†Sherds were extracted in toto.

Pottery is the essential starting point of many biomolecular archaeological investigations. Barring the recovery of discernible physical residues of natural products constituting a food or drink, pottery has the advantage of being porous and an ionic (zeolite-like) material that absorbs liquids in particular and preserves them from environmental contamination for millennia until they are chemically extracted (see below).

Pottery had some additional advantages for our study. The plasticity of the clay is ideal for producing vessel shapes suited to specific purposes, and once fired, the material is virtually indestructible. The beginning stages of pottery making in the Near East are attested at Gadachrili and Shulaveri. The pottery is well-made and functional, implying that it derives from even earlier industrial developments, possibly from a nearby mountainous region of Turkey, Mesopotamia, or Iran. Although the vessels were handmade, textile impressions on the bottoms of some bases indicated that they were probably turned on a slow wheel.

Fortunately, it has been possible to reconstruct in its entirety what is likely the principal jar type of the period. Large jars, like the one from Khramis Didi-Gora shown in Fig. 2*A*, are among the most common shapes in the pottery corpora of Gadachrili, Shulaveri, and other SSC sites. They can be very large; for example, the Khramis Didi-Gora specimen is nearly 1 m tall and 1 m wide, with a volume exceeding 300 L. Strangely, their bases, which are flattened or low disks or low pedestals, can be relatively small and seemingly unstable; the diameter of the Khramis Didi-Gora jar base is only one-quarter of its overall diameter at its widest point (*Discussion and Conclusions*).

Globules and strips of clay were sometimes applied as plastic decorations to the exterior surfaces of jars, especially very large ones. Fig. 2*A* shows 10–15 clay globules enclosed within semicircular strips at intervals around the mouth of the vessel. This motif has been interpreted as a schematic grape cluster. Small central indentations of individual globules on other jars might then represent the attachment points of bunches of berries to their pedicels. The larger knobs in the intervening spaces could indicate how a cover or lid made of an organic material (perhaps leather or cloth) was held down. Another jar from Khramis Didi-Gora is thus far unique in showing a stick-like figure with upraised arms beneath vertical lines of globules (*SI Appendix*, Fig. S1). Could this be a Neolithic rendition of a popular motif, seen on modern monuments and buildings throughout Georgia today, in which jubilant, dancing figures are seen cavorting under trellised grapevines? Chemical analysis was clearly needed as a check on any fanciful interpretations.

The pottery fabrics of all vessel types, including bowls and a range of different-sized jars with both narrow and wide mouths, are moderately well-fired, occasionally straw-tempered, and rarely polished (burnished) on their reddish-yellowish exteriors (Fig. 2 *B–D*). Interiors are generally blackish-grayish due to the narrow mouths of jars cutting off oxygen, and variously sized reduction splotches of the same colors on the exterior surfaces pointed to open-firing rather than kiln-firing (23). Interior reddish residues on the lower halves and bases of jar interiors were infrequently observed, but were suggestive of precipitates from liquid contents.

As might be anticipated, pottery was not produced on a large scale in the early Neolithic, and relatively little pottery has been recovered from these sites compared with later eras. Unlike undisturbed burials with intact vessels, human occupation, especially when a site has been intensely inhabited over centuries, usually results in whole vessels being broken into sherds and dispersed.

Based on considerations of good context and preservation, assured dating, special features such as decoration, and availability, 18 jars (6 body sherds and 12 base sherds) were sampled from the 2012–13 and 2014–2016 seasons at Gadachrili, along with one jar base sherd from the more limited 2016 season at Shulaveri. Bases were most desirable because materials settling out from a liquid were most likely to have accumulated on their interiors. Body sherds were less definitive, since they might come from the lower or upper part of a vessel. The sherds, which were not washed in the field, were accompanied by soil samples, collected from the same contexts but separated from the sherds, so as to provide a check on possible environmental contamination and background organic acid production by microorganisms.

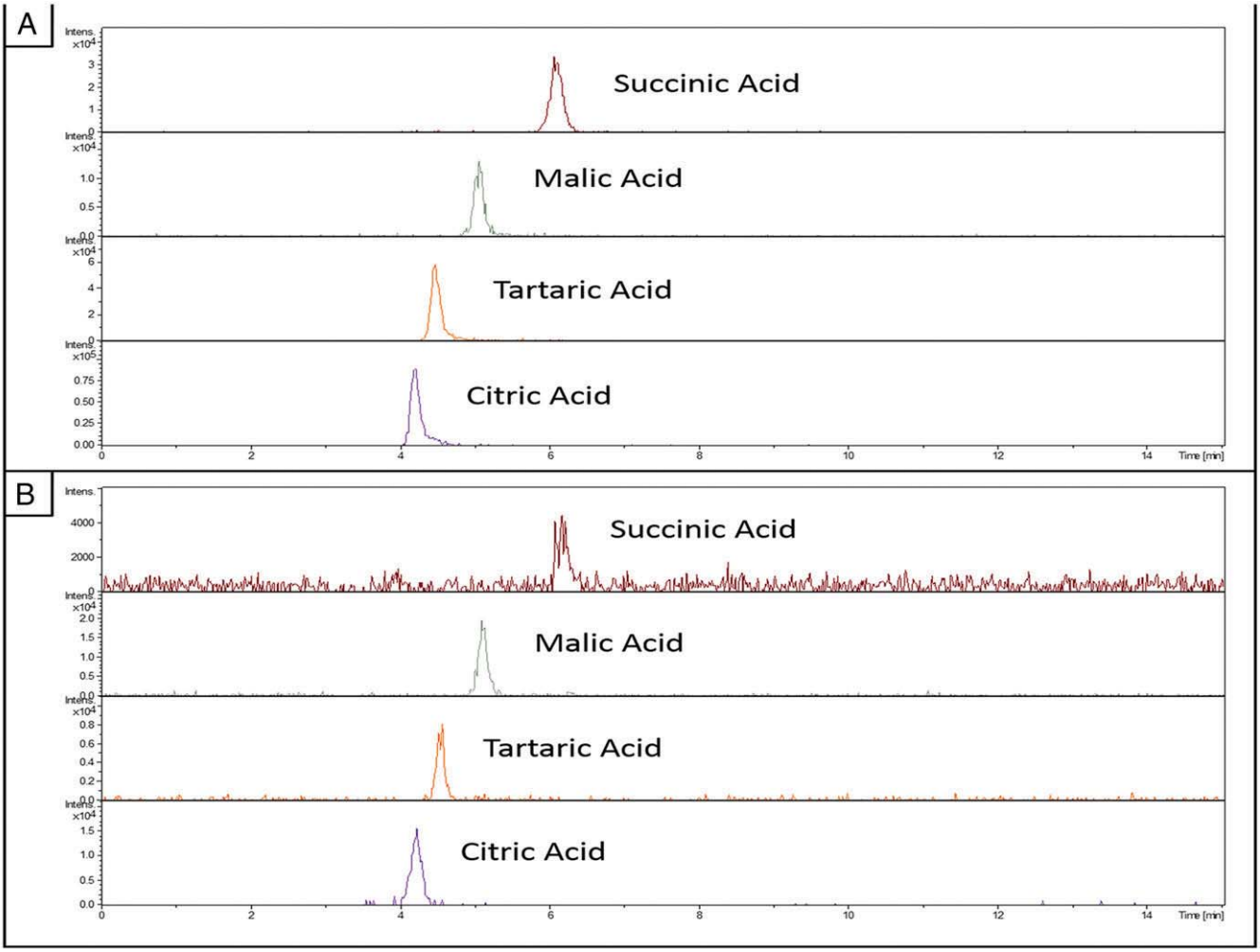

Fig. 3. Extracted ion chromatograms (±0.005-Da window) for 5 μM standard solutions (*A*), using the theoretical mass of deprotonated tartaric, malic, succinic, and citric acid, compared with jar sherd sample GG-IV-50 (*B*). All four organic acids were positively detected and quantified in this sample. Intens, intensity.

The two putative "positive" samples from the 1960s excavations at Shulaveri (one base sherd and one body sherd) were included in our analytical corpus for reanalysis with our stricter protocols and more sensitive instrumentation. Three general soil samples from Neolithic levels served as controls. Soils at both Shulaveri and Gadachrili were of the gray cinnamonic dark type.

Relative Chronology and Absolute Dating

Given our claim to have identified the earliest grape wine in the Near East (ca. 6,000–5,800 BC), it is crucial to put our findings on a solid chronological footing. Our primary reliance on short-lived botanical samples, well-defined archaeological contexts, and a Bayesian analysis of the composite data ensure that all of the analyzed samples from Shulaveris Gora and Gadachrili Gora belong to the first half of the sixth millennium BC.

Kiguradze (18) first developed a five-phase chronological model for the SSC based on the Kvemo Kartli group of sites in the Kvemo (Lower) Kartli province: Shulaveris Gora, Imeris Gora, and Khramis Didi Gora. His chronology of the relative phasing of the sites was anchored by 10 radiocarbon dates, which were carried out in the early days of the technique's development by Soviet laboratories (24). Renewed excavations at Gadachrili Gora in the same region provided an additional three calibrated dates to the corpus (21), and the 2016 excavation of Gadachrili and Shulaveris Gora added another nine calibrated dates (Datasets S2 and S3).

Even though different laboratories carried out the 22 analyses with different levels of precision and calibration, most of the dates approximated Kiguradze's original phasing and dating. A Bayesian analysis (25) of the determinations enabled Kiguradze's dates to be recalibrated with the most recent 2016 dates using OxCal v. 4. 3.2 and IntCal 13 (26–28), as shown in composite *SI Appendix*, Fig. S10 and Dataset S2. This analysis suggests that Kiguradze's fivefold model should be expanded to six phases, including an earlier phase 1 extending back into the seventh millennium BC, which is consistent with radiocarbon dates from Azerbaijan (29). Phase 1's upper limit remains to be defined by additional radiocarbon determinations.

Chemical Results

After sample extraction, ancient organic compounds were identified by a combination of chemical techniques, including Fourier-transform infrared spectrometry (FT-IR), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography linear ion trap/orbitrap mass spectrometry (LC-MS-MS) (*SI Appendix*).

Our previous FT-IR results for base sherds SG-16a and SG-782 from the excavations at Shulaveris Gora in the 1960s had been promising for the presence of tartaric acid/tartrate. In 2016,

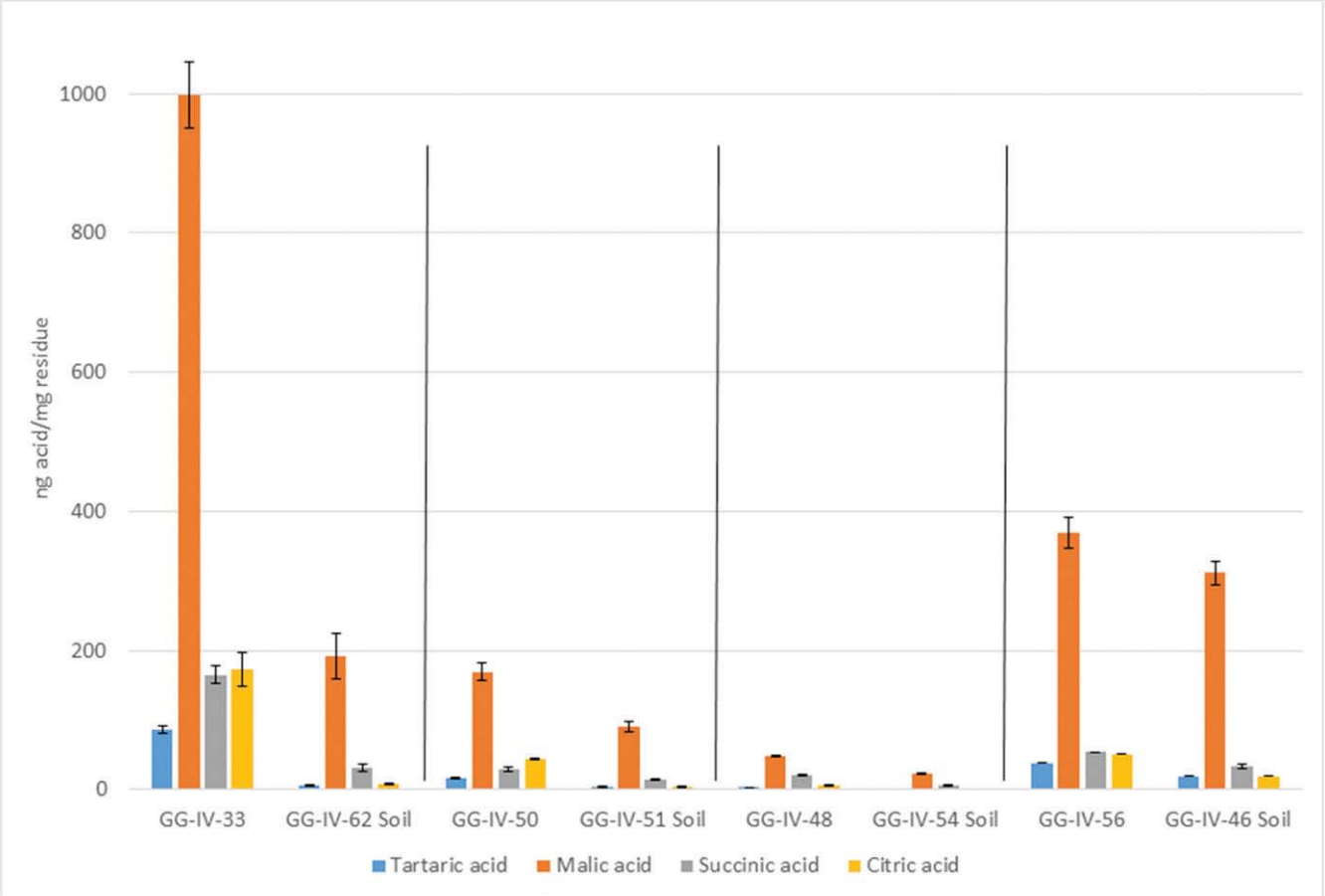

Fig. 4. Organic acid distribution for the LC-MS-MS–analyzed ancient jar base samples that were positive for tartaric acid/tartrate at Gadachrili Gora, compared with their associated soil samples. Concentrations are reported as nanograms of organic acid per milligram of extracted residue from sherd/soil material, and errors as the SD of two measurements. Note that the GG-II-9 samples (Table 1) are omitted from this graphical representation, because their data were reported as nanograms of organic acid per gram of extracted sherd/soil material.

we reran the samples, together with Neolithic soil samples from the site collected during the 2016 season. As shown in *SI Appendix*, Fig. S2, the spectrum of SG-782 had more pronounced straight-chain carbon-hydrogen stretch bond peaks at 2,920 and 2,850 cm^{-1} compared with soil, an indication that the extracted ancient sample is relatively richer in hydrocarbons. The characteristic tartaric acid doublet-carbonyl stretch bond peaks at 1,716 and 1,734 cm^{-1} were apparent for the ancient sherd, as was the hydroxyl bend at 1,452 cm^{-1}. Tartrate was identified by the carbonyl stretch bond peaks at 1,636 and 1,598 cm^{-1}, as well as the carboxylate stretch at 1,380 cm^{-1}. In contrast, the soil spectrum had very ill-defined absorptions in these regions, which might be variously interpreted.

Comparable spectra were observed for the Gadachrili sherds (e.g., Fig. 2*D*) that were positive for tartaric acid by LC-MS-MS (Table 1).

Searches of our FT-IR databases also yielded excellent statistical "matches" of the ancient spectra from both sites to those of other ancient and modern wine samples and synthetic tartaric acid and tartrate (*SI Appendix*).

Our recent GC-MS analyses were uninformative about the original contents of the jars from both sites. Fatty acids predominated in all of the samples, especially palmitic and stearic acids. The chromatogram (*SI Appendix*, Fig. S3) of jar base GG-IV-50, which was positive for tartaric acid by LC-MS-MS, is representative. Branched and unsaturated fatty acids also might occur, together with the occasional alcohol, high-numbered hydrocarbon, hopane-related triterpenoid (generic to plant cell walls), C_9 and C_{10} dioic acids (breakdown products of oleic acid), and nonspecific stigmasterol (a plant steroid). Contaminants, such as phthalate (a plasticizer ingredient of the bags in which the sherds were stored) and behenic acid (used in hand moisturizers), were ever-present.

A comparison of the chromatogram of the ancient sherd (*SI Appendix*, Fig. S4) with that of its associated soil sample (GG-IV-51) shows that the soil is richer in organics, especially high-numbered hydrocarbons (C_{27}–C_{33}) at retention times exceeding 20 min. The soil compounds are likely of modern origin. Fatty acids and n-alkanes occur widely in plants and animals, and are produced by microorganisms; they are not definitive for a grape-derived product.

The LC-MS-MS analyses proved to be most productive. Altogether, five base sherds from Gadachrili and three from Shulaveri were shown to be positive for tartaric acid and other organic acids (malic, succinic, and citric acid) found in grape/wine.

The presence of the four acids in the ancient samples is demonstrated by the exact correspondence of retention times for their extracted ion chromatograms with those of modern standards (Fig. 3). As seen in Fig. 4 and Table 1, the tartaric acid content of the positive sherds from Gadachrili (GG-II-9, GG-IV-33, GG-IV-48, GG-IV-50, and GG-IV-56) exceeded that of their corresponding background soil samples by 3.4- to 12.4-fold. At Shulaveri (Fig. 5), the tartaric acid level of SG-16a was 44 times that of the average of three Neolithic soil samples (SG-22,

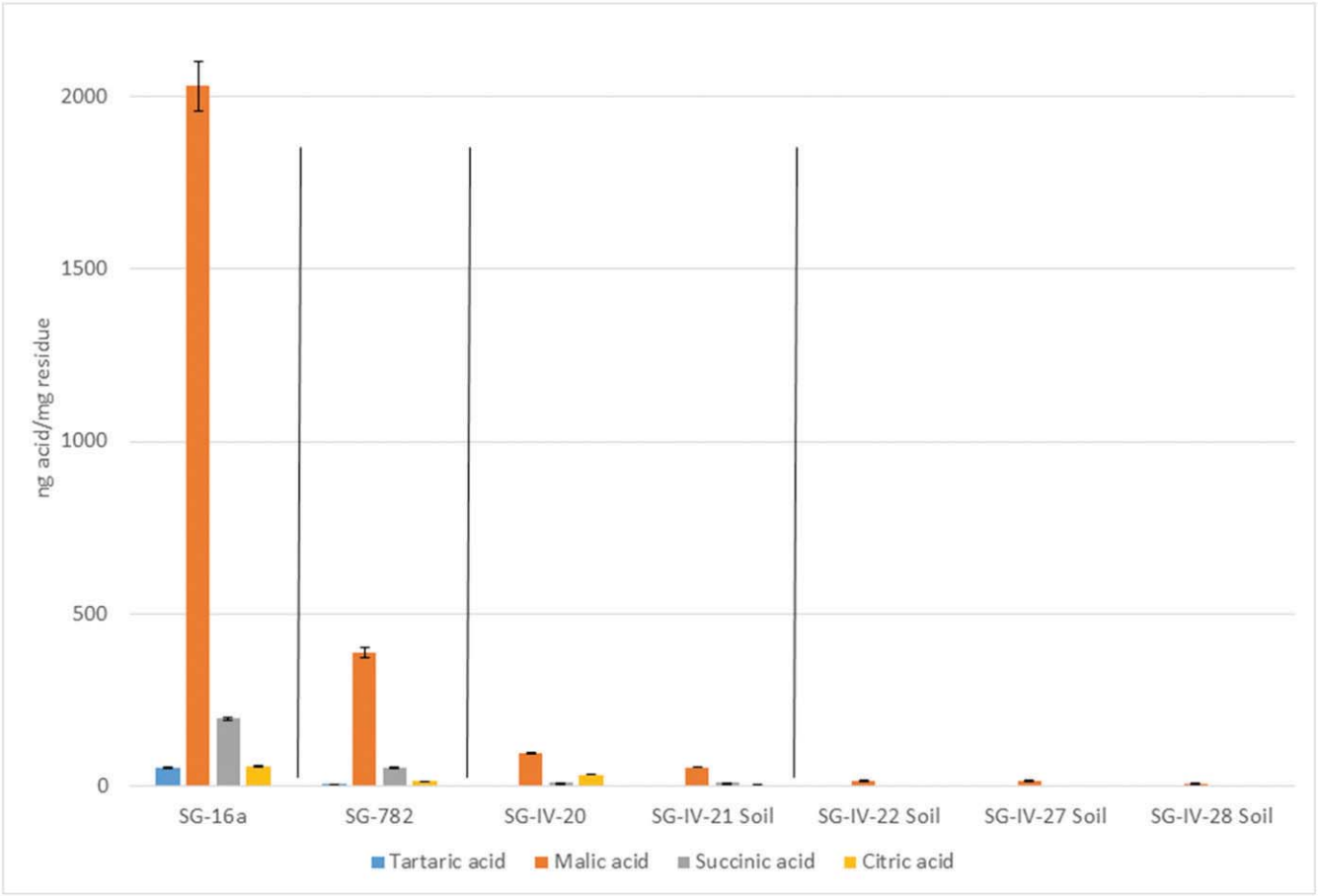

Fig. 5. Organic acid distribution for the LC-MS-MS–analyzed ancient jar base samples that were positive for tartaric acid/tartrate at Shulaveris Gora, compared with their associated soil samples. Concentrations are reported as nanograms of organic acid per milligram of extracted residue from sherd/soil material, and errors as the SD of two measurements.

SG-27, and SG-28). In contrast, the tartaric acid content of SG-IV-20 was only 1⅓ times that of its associated soil (SG-IV-21) and very low (4 ng/mg residue). Any variability in microbial soil activity (*SI Appendix*) might well lead to SG-IV-20 being classified as negative.

Negative results (not shown here) were also obtained, including 11 Gadachrili samples (five jar bases and six body sherds) with tartaric acid concentrations below those of their associated soil samples. Two other bases from this site, GG-IV-49 and GG-IV-60, did not contain any detectable levels of tartaric or the other organic acids.

Two of the bases from Shulaveris Gora (SG-16a and SG-782) were extracted as complete sherds (in toto), as was our customary procedure in the late 1990s, and were then analyzed by high-resolution LC-Orbitrap MS-MS (Table 1). The Shulaveri soils were markedly lower in abundance of the four organic acids than the soils at Gadachrili. Rainy conditions at the time of collection appear to have contributed to this difference (*SI Appendix*). High levels of tartaric acid, especially for SG-16a, provide very strong evidence for the presence of ancient grape/wine in this jar and others from Gadachrili (e.g., GG-IV-33).

Archaeobotanical Results

If grapes were exploited to make wine or used as a food source at Shulaveris Gora and Gadachrili Gora, as well as other SSC sites, then corroborative archaeobotanical evidence—seeds (pips), grapevine wood, even desiccated remains, such as skins—might be expected. Thus far, no grape pips, which have been confirmed to be Neolithic by radiocarbon dating, have been recovered from an SSC site. Those that have been excavated, including both uncarbonized and carbonized specimens, have been shown to be post-AD 1600, or "modern" in date (*SI Appendix* and Dataset S3). Only two later Middle Bronze pips were in accordance with their archaeological dating, one an uncarbonized seed with wild features per geometric morphometric analyses (ref. 24 and *SI Appendix*) from the site of Dicha Gudzuba in the port city of Anaklia and the other a carbonized pip from Pichori, north of Anaklia on the Black Sea Coast (Fig. 1), which has not yet been analyzed by geometric morphometry but appears to be of the domesticated type.

To date, the recovery of single carbonized grape pips appears to be the rule at SSC sites, including Mentesh Tepe (wild morphology; ref. 30), Göytepe (uncertain morphology; ref. 29), and Haci Elamxanli Tepe (uncertain morphology; ref. 29) in Azerbaijan. Only Aratashen in Armenia, with two pips (wild), has yielded more than one (31). Carbonized grape wood at Mentesh Tepe (30) points to grapevines growing at the site or in its environs. None of these specimens has been radiocarbon-dated, however. Possible explanations for the relative lack of grape seeds in the early Neolithic, especially given the prevalence of well-dated cereal grains from the period, are addressed below.

The archaeobotanical database for grapes at SSC sites was expanded to include evidence of pollen, starches, and phytoliths by analyzing soils and artifacts from the 2016 Gadachrili and Shulaveri excavations (*SI Appendix*). These data provide direct, contemporaneous evidence that grapes—whether wild or

domesticated is not yet clear—were an important natural resource at these sites.

Grape pollen (*SI Appendix*, Fig. S7 *A* and *C*) is widespread and abundant in many of the excavated early Neolithic contexts at both sites (e.g., locus 9 at Shulaveri; *SI Appendix*, Fig. S8*A*), but is absent from the modern top soils of the sites (*SI Appendix*, Fig. S9). The nearest grapevines in the area today are several kilometers away, and it has been demonstrated that grape pollen is distributed by wind over a short distance (32, 33). It can be concluded that the pollen from the Neolithic level is ancient. Moreover, agglomerations of pollen (*SI Appendix*, Fig. S7*A*), which are best interpreted as the remains of grape flowers, imply that grapes were growing near or even at the sites in the Neolithic. Supporting evidence for these conclusions is provided by results that are consistent with grape starch (*SI Appendix*, Fig. S7*B*) and grapevine epidermis (*SI Appendix*, Fig. S7*D*).

Pollen, palynomorphs, and nonpollen microfossils were also extracted by standard palynological analysis combined with acetolysis (*SI Appendix*) from a jar body sherd (serial no. 1828) at Gadachrili. It was excavated from a sealed context (square 10, locus 7, lot 22) inside a circular Neolithic building. Its spectrum of tree, cereal, and herbaceous pollen (*SI Appendix*, Fig. S8*B*) is similar to that of a stone grinder fragment from nearby squares 2 and 3 (locus 35). Unlike the jar sherd, however, the grinder did not yield any grape starch, grapevine epidermis, or remains of fruit flies (*Drosophila melanogaster*) (*SI Appendix*, Fig. S7*E*), which are attracted to sugar and alcohol. It can be hypothesized that the jar once contained grape wine and/or beer (compare ref. 34). Grape juice readily ferments into wine (*SI Appendix*).

Based on this microbotanical evidence, two reasonable, parsimonious inferences can be made: that grapevines were growing close to the Georgian sites, possibly inside the villages, and that their fruit was used as a food source. Combined with the chemical evidence for a grape product inside several jars, which would have served well as liquid containers, grape wine was likely one of the intended products, especially in light of the "wine culture" that emerged later in this area and throughout the Near East and Egypt.

Discussion and Conclusions

Previously, the earliest evidence for grape wine in the Near East was from the early Neolithic village of Hajji Firuz Tepe in the northwestern Zagros Mountains of Iran, ca. 5,400–5,000 BC (1, 35). Six jars, two of which were analyzed and showed the presence of tartaric acid/tartrate and a tree resin, had been embedded in the earthen floor along one wall of a "kitchen" of a Neolithic mudbrick house. Each jar when full had a volume of approximately 9 L—altogether, approximately 55 L for an average household. If that amount of wine is multiplied many times over by the houses throughout the settlement, then the production level would have already been relatively large scale at this early date. Either wild grapes were plentiful in the area or the Eurasian grapevine was already being intentionally cultivated or even domesticated. Hajji Firuz lies within the ancient and modern distribution zone of the wild grape, as established by pollen cores from nearby Lake Urmia.

The Hajji Firuz jar shapes are also well suited for vinification and wine storage, implying that they are part of an earlier industrial tradition. Their narrow, high mouths could have been stoppered with clay (some possible examples with the same diameter as the mouths of the jars were found nearby) or covered.

Hajji Firuz is only approximately 500 km from Shulaveri and Gadachrili, and even closer to sites in Armenia and Azerbaijan. These sites also lie within the zone of the wild grape, as does the mountainous region of northern Mesopotamia and, farther afield, the Taurus Mountains of eastern Anatolia. Now that wine jars from as early as ca. 6,000 BC have been confirmed for Gadachrili and Shulaveri, preceding the Hajji Firuz jars by half a millennium, the question might be asked which region has priority in the discovery and dissemination of the "wine culture" and the domesticated grape. It is impossible to assign priority to any of these regions at this stage in the investigation; much more excavation and the collection of wild grapevines for DNA analysis are needed.

One disparity between the analyses of Hajji Firuz and Georgian jars is that the latter showed no signs of a tree resin or any other additive, according to the GC-MS analyses. Pine and terebinth saps were commonly added to wine throughout antiquity. They acted as antioxidants to keep the wine from going to vinegar, or barring that, to cover up offensive aromas and tastes. The tradition continues today only in Greece as retsina.

The Hajji Firuz jars were found partly buried in an earthen floor. No evidence has yet been found of how the Shulaveri and Gadachrili jars were positioned or whether they were partly or fully buried underground, as is the common practice for making so-called *qvevri* ("large jar") wine today in Georgia. The very small, flat bases of the ancient jars, often disks or low pedestals, seem inadequate to independently support a vessel full of liquid, so a case could be made for burying them. But then why even provide them with such unstable bases, unless these were decorative like the plastic decorations on some examples?

The earliest archaeological evidence for *qvevri* winemaking in Georgia is Iron Age in date, specifically the eighth to seventh centuries BC By Roman and Byzantine times, *qvevris* had become very popular throughout the Near Eastern and Mediterranean worlds; for example, excellent examples have been unearthed at Pompeii. Strangely, however, no examples of large jars buried underground like those at Areni in Armenia have been found in Georgia for the 5,000-y period from the Neolithic period to Iron Age times.

Based on ancient Egyptian frescoes, the earliest pictorial record of winemaking in the world, fermenting wine in medium-sized jars (amphoras) totally above ground was the preferred method since ca. 3,000 BC (1, 36). Given that Canaanites introduced viticulture, winemaking, and the amphora ("Canaanite jar") to Egypt, it can be assumed that they performed vinification and storage of wine, as the Phoenicians did later, in the same way.

The breakthrough came when numerous underground jars were found inside caves at Areni in a mountainous region of Armenia (37). Desiccated (uncarbonized) grapevine wood, dating to ca. 4,000 BC, together with pips and chemical evidence by LC-MS-MS of tartaric acid/tartrate and the red pigment malvidin, left no doubt that we now had partial evidence for the previously "empty" transitional period. The technology was ingenious: humans had laid out plaster floors for pressing the grapes and running the unfiltered juice into underground jars. Whether similar evidence will eventually be found in Georgia and Azerbaijan, elsewhere in the SSC area, or in the extended mountainous region remains to be seen.

The prominence of cereals in the early Neolithic SSC sites was likely due to a combination of factors. Barley and the wheats (einkorn and emmer) were domesticated very early in the Near East, perhaps by ca. 10,000 BC. They provided the all-important ingredients for beer and bread, staples that were produced in quantity in succeeding periods. The probable later domestication of the grapevine, combined with the fact that it takes a minimum of 3 y to establish a vine to bear fruit, meant that grapes would have been a rarer commodity than grain.

What makes the domesticated vine so desirable for larger-scale production is that it is hermaphroditic, with both the male and female reproductive organs contained within a single flower, where fertilization readily occurs. The wild vine is dioecious, with separate male and female plants, so that it is dependent on the wind and, to a lesser extent, insects for pollination. Only a portion of the wild vine population—the female individuals—can produce fruit, and even then, not all flowers are pollinated. Consequently, wild vines produce far less fruit than domesticated vines.

Wine making also does not make direct use of the seeds, as do beer making and bread making. Because of their bitterness, pips were usually considered waste to be discarded. In contrast, whole, unprocessed cereal grains in a bread or beer are not necessarily detrimental to the end product, and might even be considered to provide more body and taste.

Grape pressing and winemaking were generally done near where the grapes grew in antiquity, to avoid heavy transportation and conserve space within the settlement. The dense concentration of circular buildings at Shulaveri and Gadachrili would have left little room for growing grapes. Small numbers of pips might have made their way to the bottoms of the wine jars, to be disposed of later within the settlement. To date, however, no jar with seeds has been recovered from an SSC site.

Moreover, bread making and beer making require heating installations for the best results. Simply placing a mixture of ingredients under a hot sun can work, but is less reliable and efficient. Open firings around jars for beer mashing (saccharification of grain starches into sugars for fermentation) have been excavated in proto-Dynastic Egypt, ca. 3,500 BC (2, 38). Pit-firing installations associated with flat stones for possibly drying, malting, and/or baking bread or making beer are attested as early as the Pre-Pottery Neolithic period, ca. 8,700–6,500 BC, in the Near East (39). Even earlier firing installations, associated with barley starch embedded in a basalt grinding stone, have been excavated at Ohalo II, located along the southwestern shore of the Sea of Galilee and dating to the Epipaleolithic period, 23,000 y ago (40, 41). Eurasian wild grape seeds also have been reported from this site (40). Inevitably, if the processing of cereals for bread, beer, and/or another product was done nearby, some grains might have fallen into the fire or been overheated, and thus carbonized. Spent cereal grains might also have been used as fuel.

Grape fermentation does not require a heat source; in fact, a cool environment, such as a cave or burying jars underground, is best. We can conclude that bread making/beer making and winemaking occurred in different places in ancient sites, the former of which contributed to the production of masses of carbonized grains, which are well-preserved, and the latter of which resulted in low amounts of carbonized seeds.

Cereals could be dried and stored in a settlement for easy use when needed throughout the year. Grapes could be dried as raisins, but like uncarbonized pips, they generally degraded and have disappeared from the archaeological record. Grapes also can be preserved by concentrating them down into a syrup, but if this was the intended product, then pottery vessels from the SSC sites should show signs of carbon splotches due to exposure to fire on their exteriors. None do.

These considerations lead to the conclusion that the jars excavated at Shulaveri and Gadachrili, which provide chemical and archaeobotanical evidence for grape, probably originally contained wine. If their contents were high enough in alcohol, they would have provided much more than year-round sustenance for early Neolithic inhabitants. Much like Georgia's wine culture today, wine likely also served as a medicine, social lubricant, mind-altering substance, and highly valued commodity. As such, it became the focus of religious cults, pharmacopoeias, cuisines, economies, and society in general.

This "working hypothesis" (22), while buttressed by new archaeological, chemical archaeobotanical, and climatic/environmental data, is only a beginning. We may now have evidence that at least two SSC sites in Georgia, Shulaveris Gora and Gadachrili Gora, were making grape wine as much as a half millennium earlier than Hajji Firuz Tepe in Iran. However, many other regions of the Near East, especially the broad arc of mountainous terrain bordering the Fertile Crescent on its north, remain to be investigated and studied scientifically.

Thus far, we have focused on jar residues from the Pottery Neolithic period, but a Pre-Pottery period preceded it, going back to ca. 10,000 BC. During the ensuing four millennia, the first permanent settlements, sustained by the founder crops, were established. Sites of this period are yet to be discovered and excavated in the SSC region of eastern Georgia, but they are well represented westward and southward in other mountainous regions.

With their extraordinary monumental architecture and artwork, Göbekli Tepe (42) and Nevali Çori (43) in the Taurus Mountains of southeastern Anatolia stand out among Pre-Pottery Neolithic sites. The domestication of three founder plants—einkorn wheat (44), chickpea, and bitter vetch—has been traced to this region. It has been proposed that wheat to make beer was the incentive that drew humans here and led to the grain's domestication. Fermentation might have been carried out in large limestone vats at Göbekli Tepe, which are the focus of ongoing chemical analyses (42). Stone bowls and goblets have also been excavated at the sites; as precursors of examples in pottery, and they were ideally suited for serving and drinking a fermented beverage. Chlorite, the stone they were made of, is a highly absorbent clay mineral that retains ancient organic compounds like pottery. The vessels are now being extracted and chemically analyzed (45).

But did the people of Göbekli Tepe and Nevali Çori limit their alcohol quaffing to wheat beer? Perhaps they experimented with wild Eurasian grape wine or honey mead. We hope to learn more about the beginnings of viniculture by the careful excavation of more archaeological sites, the fullest recovery of the micro and macro remains of our largely lost and destroyed past, and the application of the most exacting scientific techniques.

Finally, it should be noted that Jiahu in the Yellow Valley of China still has the distinction of having produced the earliest chemically confirmed grape wine in the world, as early as ca. 7,000 BC (46). This wine was probably made from a local, high-sugar wild species there. However, this early Neolithic fermented beverage was not purely a grape wine, like that in the South Caucasus appears to have been, but was combined with hawthorn fruit wine, rice beer, and honey mead.

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Influence of climate cycles on grapevine domestication and ancient migrations in Eurasia

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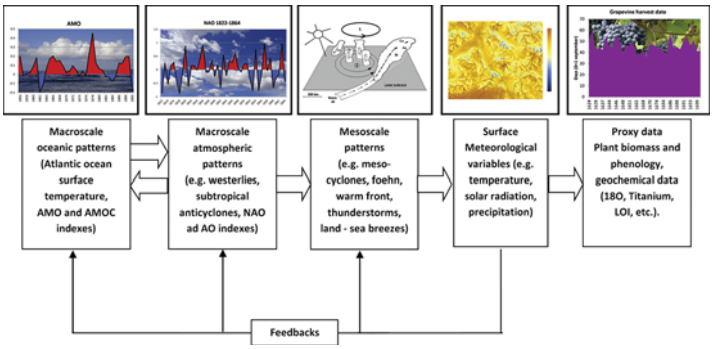
HIGHLIGHTS

- Detection of cycles in atmospheric, oceanic and astronomical forcing series
- Detection of cycles in geochemical and biological proxies
- Establishment of possible causal relations between forcing and proxies
- Analysis of the cyclical events in the Western and Eastern part of the studied area
- Deductions on the domestication and expansion of viticulture from Caucasus area towards current production areas

GRAPHICAL ABSTRACT

Description of the causal links between all-scale forcings and proxy data. The feed-backs between microscale and upper scales are highlighted. These links mask a massive flow of matter, energy and information between scales.



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ABSTRACT

The objective of this work is to investigate the Holocenic climate cycles that may have influenced the domestication of grapevine in the Subcaucasian area and its subsequent spread in Eurasia. The analysis covered the longitudinal belt ranging from the Iberian Peninsula to Japan, seen as the preferential pathway for the Holocenic spread of grapevine and many other crops in Eurasia. Spectral analysis was considered as the criterion of investigation and the Holocenic cycles were analyzed considering different geochemical and biological proxies, of which seven are directly referred to vine. In this context the relation of the abovementioned proxies with spectral peaks of possible causal factors like Solar activity (SA), North Atlantic oceanic factors (Atlantic Multidecadal Oscillation - AMO and North Atlantic Oscillation - NAO), and subtropical oceanic factors (El Nino Southern Oscillation - ENSO) was also analyzed.

In order to acquire a sufficiently wide number of proxies sensitive to the causal factors, we referred to a latitudinal belt wider than the one colonized by vine, also acquiring proxy from the Scandinavian area, notoriously susceptible to North Atlantic forcings. The analysis of the proxy spectral peaks, considering 20 classes with a 50-years step in the 0–1000 years range, showed that the 50% of the classes have a higher frequency of peaks at East than West, the 20% a higher frequency at West than East and the 10% an equal frequency, showing the efficiency of the propagation of Western signals towards the center of Eurasia.

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The search of the causal factors spectral peaks in the proxy series showed that AMO, NAO and SA acted with a certain regularity on the entire belt investigated both latitudinally and longitudinally, while spectral peaks linked to ENSO underwent a considerable attenuation moving northward.
Finally, the specific analysis on viticultural proxies showed common peaks with causal factors.

1. Introduction

1.1. Climate and grapevine history

The appearance of the *Vitis* genus to which the cultivated grapevine (*Vitis vinifera* L.) belongs has to be dated back to 60 million years ago, in the very hot Eocene. The following events brought the *Vitis* genus to be made up of over 60 species, one of them being *Vitis vinifera sylvestris*, the wild ancestor of domestic vine, which occupied a large territory between Western Asia and Euro-Mediterranean area. Two million and a half years ago, the glacial eras of the Quaternary began, and during that time the vine, as a thermophilic species, limited its presence to the coastal strip overlooking the Mediterranean and in refuge areas southern of large mountain ranges (Great Caucasus, Alps, Pyrenees). It was probably here that, during the Würm glaciation, humans came into contact with grapevine, eating its fruits and maybe producing wine ancestors fermented drinks.

About eleven thousand years have passed since the beginning of the Holocene, which saw the birth of agriculture in some main domestication centers (East Asia, Sub Sahara, Central and South America and Middle East) (Gepts, 2004). More specifically the Middle East was theater of the domestication of many crops like wheat, barley, peas, chickpeas and grapevine. While domestication of first cereals was deeply studied, the origin of cultivated grapevine is still less known, in spite of the efforts to make progress in this field. About that, one of the most intriguing questions the researchers were asked to answer was how many millennia the first wine came from. McGovern et al. (1996) located this event at 7000–7400 years Before Present (BP) on the base of biochemical analysis of the traces of vinification present in jars found in Hajji Firuz Tepe on the mountains of Zagros (Iran). A more recent research (McGovern et al., 2017) investigated the traces of vinification dated 8000 years BP on archaeological remains excavated at the Neolithic sites of Shulaveris Gora and Gadachrili Gora, located in the alluvial plain of the Kura River 50 km South of the modern capital of Georgia Tbilisi and belonging to the "Shulaveri-Shomutepe culture" (about 8000 BP) (Batiuk, 2013). This work shifts 600–1000 years back than previous finds the first winemaking event and paleoclimatic analysis showed that climate of that period was similar to the current one for thermal and pluviometric resources. However, the abovementioned archaeobotanical evidences of the extraction of grape juice cannot be considered proofs of domestication or even cultivation of grapevine. Additionally, no indication about cultivation is dated before the 4th–3rd millennia BCE (Terral et al., 2010).

As other crops domesticated in the Fertile Crescent grapevine migrated along the mid latitude belt both Westward (North Africa e Western Europe) and Eastward, towards China and Japan, along the complex network of land trade routes presently called Silk Road.

Westward migration involved firstly the Mediterranean climate areas (Csa type of Köppen Geiger classification). So, 4500 years BP grapevine arrived to Greece and Egypt and 3000 years BP to Italy while grape cultivation in Southern France began with the foundation of the Greek city of Massalia (the modern Marseille) 2600 years BP (Terral et al., 2010) concurrently with the arrival in the Iberian Peninsula due to Greeks or Phoenicians (Buxó and Capdevila, 1997). Only later, during the Roman Empire, grapevine reached the oceanic climate areas of Europe (Cfa type of Köppen Geiger classification) like Atlantic France, Germany and England (Brown et al., 2001). Later, the Medieval Climatic Optimum (MCO) caused a latitudinal and altitudinal expansion of vine crop area, while a new reduction took place during the Little Ice Age (LIA) (Behringer, 2009).

Slower was the expansion of domestic grapevine Eastward. For example in Japan the first culture of *Vitis vinifera* dates probably back to the 10–12th century (Morinaga, 2001).

During this long and complex series of events that cover about eight thousands years, grapevine genotypes constantly interacted with the European climate and its variability leading to the modern viticulture both in terms of plant distribution and genotype selection.

Thus, we come to the recent expansion of grapevine towards oceanic environments with large number of rainy summer days, made possible by the improved pest and disease management techniques, and towards environments with low precipitation, thanks to improved irrigation techniques. In this way grapevine colonized new continents (America and Australia).

1.2. The main features of the Eurasian climate at mid latitudes

To give an overview of the macroscale features of the Eurasian climate an ideal point of departure is given by the zonal regime that is characterized by occidental currents (Westerlies) that flow between the subtropical anticyclones belt and the high latitudes low pressure belt. With a zonal regime, the mild polar maritime air is advected towards Europe from the Atlantic Ocean. The zonal regime is periodically broken by blocking patterns (Charney and DeVore, 1979; Holton, 2004), causing the advection of (i) hot subtropical air masses from subtropics or (ii) cold arctic air masses from inside the polar circle or (iii) polar continental air from Siberia. This peculiar behavior is resumed by the NAO (North Atlantic Oscillation) index, a large scale feature of natural climate variability with important impacts on the weather and climate of the North Atlantic region and surrounding continents (Knudsen et al., 2014). NAO is given by the normalized difference of surface pressure between a southern station (e.g. Lisbon or Gibraltar) and a Northern one (e.g. Rejkiavic). On the other hand, paleoclimate NAO time series are usually rebuilt on the base of co-variability of precipitation-sensitive proxies (Trouet et al., 2009) or temperature-sensitive proxies (Franke et al., 2017b).

Since NAO is particularly effective on winter Eurasian climate, the NAOI index, the average of NAO Index over the December to March period (Osborn, 2000), is commonly adopted. More specifically, a positive value of the index is favorable to advection of mild and moist maritime air masses from the Atlantic towards Europe while advection of polar continental air is observed with a negative NAOI (Hurrell, 1995).

It is the change in frequency and persistence of various atmospheric circulation regimes and therefore among the different air masses that give the most important imprinting to European climate, whose features are also modulated by (i) the effects of mesoscale patterns such as Mediterranean lows or föhn/stau patterns or mobile anticyclones and (ii) the surface temperature of the Atlantic Ocean described by the index AMO (Atlantic Multidecadal oscillation) (Kilbourne, 2014). AMO is driven by changes in the Atlantic Meridional Overturning Circulation (AMOC) (Buckley and Marshall, 2016) and its influence is advected over lands by the atmospheric circulation. AMO shows cycles influenced by NAO activity because a positive phase of the NAO strengthens the AMOC by extracting heat from the subpolar gyre, thereby increasing deep-water formation, horizontal density gradients, and the AMOC (Delworth and Zeng, 2016). In this context some years of strongly positive NAO triggers the AMO transition from negative to

positive values (McCarthy et al., 2015) with a delay of about 5 years which are needed to spread the new phase thorough the North Atlantic (Knudsen et al., 2014).

The AMO index activity, documented since 1857 by direct measurements, is also testified for the last 8000 years by a multiproxy approach (Knudsen et al., 2011). As implied by the term "Oscillation", the AMO is subject to characteristic cycles with relevant effects on the thermal and pluviometric regime of the Euro - Mediterranean area (Sutton and Dong, 2012).

The abovementioned scheme is described in graphical abstract showing (i) the contribution of forcing at different scales to surface meteorological variables and (ii) the feedback contributions of lower scales to upper scale facings. In their turn, surface meteorological variables are the main forcing for a cascade of abrupt environmental changes that imprint the various kinds of proxy data considered for this work. An example of this causal chain referred to the '80s regime shift is reported by Reid et al. (2016).

1.3. Climate cycles and viticulture

Focusing on climate cycles, within the quaternary the glacial-interglacial alternation had a characteristic period of 10^5 years while the interglacial climate of the Holocene (last 11 thousand years) provides many examples of climate cycles on scales of millennia, centuries, decades and years (Mariani and Zavatti, 2017). More specifically in mid latitudes of the Boreal Hemisphere climate cycles can be classified into:

short period cycles (few years) resulting from short-term periodic phenomena such as El Niño Southern Oscillation - ENSO, Quasi Biennial Oscillation - QBO (Kutzbasch and Bryson, 1974), Mediterranean Oscillation Index - MOI (Conte et al., 1989) and NAWA (Paz et al., 2003).
long period cycles, determined by causal factors only partially known such as solar cycles (SA), the Atlantic Multidecadal Oscillation (AMO) and the North Atlantic Oscillation (NAO).

Cyclic components significantly affect productivity and quality of grapevine production with strong effects on viticultural systems as stated for example by Tourre et al. (2011), with sequences of bad years (hot and dry periods, cold and rainy periods and so on) that may even lead to the abandon of viticulture in disadvantaged areas or, conversely, sequences of favorable years, driving the expansion of viticulture towards new territories. Such effect was probably more relevant in the past when the adaptation to climate variability was not supported by modern technologies (improved varieties, rootstocks, fungicides, pesticides, wine production techniques, mechanization of planting and management of vineyards and so on), as depicted by Emmanuel Leroy Ladurie, when he compared human civilization to a war against the dictatorship of climate (Vasak, 2010).

Following this basic scheme, we can interpret the answer of viticulture to mild phases (e.g. the Holocenic, the Mycenaean, the Roman and the Medieval Climate Optimums, HCO, -MyCO, RCO and MCO respectively) and cold periods (e.g. the iron age deterioration and the Little Ice Age - LIA) (Behringer, 2009).

Another important and yet open question is how differently climate variability affected the Western and Eastern part of the Mediterranean basin.

By a documental point of view, a first exhibit comes from Ovid who, during his exile in the little harbor of Tomis (now Constanta, RU) on the Black Sea, complained about the bad cold climate, so different from the Italian one. Since today Tomis climate shows Mediterranean traits, the difference highlighted by Ovid could be seen as a trace of the Eastern delay in the beginning of the Roman climatic optimum, already affecting the Western part of the Mediterranean basin.

Some insights about the question of West-East delay were proposed by Cola et al. (2016), discussing the thermal effect of the change of phase of NAO (from negative to strongly positive in 1987) and the consequent change of phase of AMO (from negative to strongly positive in 1994). In Western Europe, temperature immediately raised in 1987 with an average increase of about 1 °C in mean yearly temperatures while Georgia answered later in 1994 with an average increase of +1.4 °C. Moreover, the same phenomenon observed for Georgia was highlighted by Ghanghermeh et al. (2015) for North Iran.

On the other hand, Messager et al. (2017), analyzing pollen series from Nariani (Georgia), stated a strong delay in the postglacial forest expansion compared to Western Europe and this phenomenon was explained by the authors as an effect of the Black Sea and the Caspian Sea fulfilled of cold water coming from the glacial ice melting. This set of phenomena referring to very different time scales could be interpreted as effects of the higher degree of continentality (Oliver, 2005) that characterizes the climate of Eastern Europe, caused by the higher distance from the Atlantic Ocean and its strong climatic signals, as stated by physical geographers (Shahgedanova, 2002).

In the light of the abovementioned evaluations the main aims of this work are:

to detect the presence of cycles in some atmospheric, oceanic and astronomical forcing series for the geographical belt extending from Iberian peninsula to the whole Eurasia
to detect the presence of cycles in geochemical and biological proxies in the same area
to use the cycles to establish possible relations between forcing and proxies
to analyze the existence and strength of discrepancies between cyclical events in the West and East part of the studied area
to made deductions for domestication and expansion of viticulture from Caucasus area towards current production areas.

2. Data

The time-series analyzed in this work were divided in two groups, the first gathering the causal factors of climate cycles (Table 1) and the second the proxy series supposed to be affected by these factors (Table 2). With reference to the first, AMO - a temperature indicator of the surface of the North Atlantic, is represented by instrumental series 1 and by long-time proxy-derived series 2. Series 12 is the merged series of 1 and 2, used for analysis purposes. NAO - an indicator of the intensity of the Westerlies carrying towards the East the signals that are generated on the Ocean, is represented by instrumental series 3 and by long-time proxy-derived series 4. Series 13 is the merged series of 3 and 4, used for analysis purposes. Series 5, ENSO-Nilometer that reports the Nile River's water level during the annual flood season which is a proxy of the activity of the Indian Ocean monsoon because blue Nile is fed by monsoon rain falling on the Ethiopian highlands. Moreover, the Indian Ocean monsoon is related to ENSO, because Monsoon use to slow down during ENSO years (Parisi et al., 2018). Series 6 is a reconstructions of the solar activity based on ^{10}Be and ^{14}C (Wanner et al., 2008).

In order to attain a more comprehensive view of the causal relations between forcings and proxies, the following teleconnective indices were analyzed:

the Mediterranean Oscillation Index MOI (7) which is the normalized pressure difference between Algiers and Cairo calculated for the period 1948–2016 (Climate Research Unit, 2017)
the East Atlantic Pattern EA (8) which is structurally similar to the NAO (north-south dipole of anomaly centers spanning the North Atlantic from east to west) and is calculated for the period 1950–2017 (NOAA, 2018a)
the East Atlantic/West Russia Pattern EAWR (9) which is referred to four main anomaly centers (Europe, Northern China, central North

Atlantic and North of the Caspian Sea) and is calculated for the period 1950–2017 (NOAA, 2018a)
the Polar-Eurasia Pattern POL (10) which is referred to height anomalies over the polar region, and over northern China – Mongolia and is associated with fluctuations in the strength of the circumpolar circulation calculated for the period 1950–2017 (NOAA, 2018a)
the Scandinavia Pattern SCA (11) which is referred to a primary circulation center over Scandinavia, with weaker centers of opposite sign over western Europe and eastern Russia/western Mongolia and is calculated for the period 1950–2017 (NOAA, 2018a).

The time series of MOI, EA, EAWR, POL and SCA are currently too short, covering only periods of <70 years. For this reason, only AMO, NAO, ENSO and Solar Activity were taken into account in our analysis. Obviously, the analysis could extend to those and other indices whenever their paleoclimatic reconstructions will be available.

The cycles affecting the aforementioned causal factors are then sought in the time series of physical and biological data that are presumed to carry trace of solar, atmospheric and oceanic signals. Time series of proxy data are listed in Table 2 where they are characterized in terms of type of proxy analyzed, geographical features (country, longitude, altitude) and Köppen-Geiger classification of their present climate. Among the 38 selected time series of proxy data, 25 belong to group C (temperate/mesothermal), 6 to group B (desert and semi-arid), 7 to group D (continental/microthermal) and 2 to group E (polar).

3. Methods

The spectral analysis of the collected time series was based on the two main analytical methods Maximum Entropy Method (hereafter MEM; Childers, 1978) and Lomb-Scargle Periodogram (hereafter LOMB; Lomb, 1975; Scargle, 1982).

The MEM method was applied on evenly-spaced data. We followed what published in Press et al. (2009) and used the highest allowed pole order, which is half of the length of the record. Such technique can resolve distinct sinusoids (i.e. sharp spectral features) and, on the other hand, gives rise to spurious peaks in the flat noise background.

The LOMB method was applied when the series are unevenly spaced and in general when they have missing data. Again we followed what Press et al. (2009) made available, with *ofac* = 5 and *hifac* = 4 as normal setting, *hifac* = f*hi*/f*c* being the ratio between "how high in frequency to go, or f*hi*" and the Nyquist frequency f*c* and *ofac* as an oversampling parameter which allows a better sampling at finer intervals than 1/T, T being the span of the input data or the frequency such that the data can include a complete cycle. When and if it would be necessary, ofac and hifac were both lowered by 1 (4 and 3 respectively). The Lomb method requires a preliminary data detrending carried out computing linear trends by a least square fit and subtracting them to the data.

As far as the confidence levels of spectra are concerned, we note that the so-called "peaks affected by or embedded into the noise" are such when a comparison with the spectral maxima of other independent series cannot be performed: when a period (also a low-power one) can be confirmed by the spectra of several other time series we assume it could be real, no matter what its amplitude/power might be.

In order to approach the analysis of Western and Eastern side of the study area we divided the proxy time series in Western (<25° of longitude) and Eastern series (≥25° of longitude) and tested how the frequency of positive peaks varied between West and East. Even if we realize that the ideal divide between East and West was given by the probable area of domestication (35° E), the choice of 25° E is justified by the need to have a balanced number of samples between West and East.

To test the significance of the differences between West and East spectral signals, a two-tailed Student's *t*-test was applied to the percent frequencies of the number of the spectral maxima, assuming a Poisson distribution of the maxima. The analysis of spectral peaks was addressed only to peaks above 10 years, which are more difficult to constrain because they are outside the short term variability that the vine-grower is used to account for and generally able to deal with without particular adaptation efforts. A possible exception to this general rule is given by the very cold and rainy year 1740 (Leroy Ladurie, 2006), particularly ruinous for French agriculture (traditionally based on grapevine and winter cereals) because it followed a long sequence of mild and favorable years.

In order to deepen the coherence among the causal factors and the proxy series, four causal series were considered (ENSO_Nilometer, Solar activity, AMO_syn and NAO_syn). For each selected causal factor, all the proxy series were separately analyzed. For each proxy series only the causal factor spectral peaks falling into the period range of the proxy series were taken into account. For instance, in the case of causal factor NAO and proxy series Morocco TR, the Morocco TR series (periods: 550, 241, 175, 96, 65, 48, 34) covers the range 0–550 years and 10 NAO periods out of 11 were considered, while period 695 was discarded (over 550 limit). For each of the 10 NAO periods a confidence interval of ±20% was considered in order to check if Morocco periods fall into the interval. So with reference to NAO period 36, the interval 28.8–43.2 was obtained. The Morocco period 34 falls into this interval, giving a positive check between NAO and Morocco TR. Globally, the Morocco TR series is positive in 6 cases out of 10 NAO periods, giving a final result of 60% of coherence. The same test was performed for each proxy series for all the four causal factors.

4. Results

Tables 3 and 4 show the detection of peaks up to 15,000 years for causal factors and proxies respectively. The results of the spectral

Table 1
Time series of selected causal factors.

n.	Time series	Time period	Data type	Data source
1	AMO	1856–2016	Instrumental data	NOAA, 2018c
2	AMO proxies	500–2000	Model reconstruction	Mann et al., 2009
3	NAO	1856–2016	Instrumental data	NOAA, 2018b
4	NAO proxies	0–2000 CE	Proxies from Greenland, Alps and Fennoscandia	Franke et al., 2017a, 2017b
5	ENSO-Nilometer	622–1469 CE	Annual minimum level of Nile	Hipel and McLeod, 1994
6	Solar activity	8000 BCE – 2000 CE	solar activity based on 10Be and 14C	Wanner et al., 2008 (Fig. 8), Scafetta, 2012
7	MOI	1948–2016	Mediterranean Oscillation Index	Climate research Unit, 2017
8	East Atlantic Pattern EA	1950–2017	Instrumental data	NOAA, 2018a
9	East Atlantic/West Russia Pattern EAWR	1950–2017	Instrumental data	NOAA, 2018a
10	Polar-Eurasia Pattern POL	1950–2017	Instrumental data	NOAA, 2018a
11	Scandinavia Pattern SCA	1950–2017	Instrumental data	NOAA, 2018a
12	AMO_syn	500–2016	Instrumental & proxy data	AMO series obtained merging AMO (NOAA, 2018c) and AMO (Mann et al., 2009) proxies
13	NAO_syn	0–2016	Instrumental & proxy data	NAO series obtained merging NAO (NOAA, 2018b) and NAO proxies (Franke et al., 2017a, 2017b)

Table 2
Time series of proxy data listed by growing longitude.

n.	Time series	Alt	Time period	Data type	Data source	Lon	Lat	Country	Rep_T*	Rep_RR*	Climate
1	Morocco TR	>2100	1000–2000	Tree rings	Esper et al., 2007	−5.00	33.00	Morocco	L	H	Csb
2	Medoc GHD	50	1752–2006	Grapevine harvest dates	Chevet and Soyer, 2006	−0.35	44.51	France	H	L	Cfb
3	Lake Redon T	2080	578–1994	Temperature rebuilt from chrysophyte cysts from lake sediment	Pla and Catalan, 2004	0.93	42.62	Spain	H	N	Cfc
4	Burgundy C GHD	350	1370–2010	Grapevine harvest dates	Chuine et al., 2004	4.23	47.00	France	H	L	Cfb
5	Burgundy D GHD	350	1354–2006	Grapevine harvest dates	Daux et al., 2012	4.23	47.00	France	H	L	Cfb
6	Burgundy T GHD	350	1676–2004	Grapevine harvest dates	Tourre et al., 2011	4.23	47.00	France	H	L	Cfb
7	Beaune GHD	200	1371–2010	Grapevine harvest dates	Labbé and Gaveau, 2013	4.50	47.00	France	H	L	Cfb
8	Lake Verney LOI	2188	0–11 kyrs BP	LOI	Bajard et al., 2017	6.53	45.41	Swiss	H	L	Dfc
9	Lake Folgefonna ELA	938	19–5932 BP	Glacier ELA from lake sediment data	Bakke et al., 2005	6.59	60.21	Norway	H	M	Cfb
10	Lake Bachalpsee LOI	2265	0–12.5 kyrs BP	LOI	Lotter et al., 2006	8.01	46.40	Swiss	H	L	Dfc
11	Swiss Plateau CFD	900	1721–2012	Cherry (*Prunus avium* L.) flowering dates	Rutishauser, 2008	8.30	47.00	Swiss	H	L	Cfb
12	Swiss plateau GHD	900	1624–2003	Grapevine harvest dates	Meier et al., 2007 Niemann	8.30	47.00	Swiss	H	L	Cfb
13	Lake Cadagno T	1921	0–11 kyrs BP	Temperature rebuilt from bacterial membrane lipids Grapevine	et al., 2012 Mariani et al.,	8.71	46.55	Swiss	H	N	ET
14	Tirano GHD	600	1624–1930	harvest dates	2009	9.52	46.10	Italy	H	L	Cfb
15	Iberian Margin SST C37 16	0	0.4–31.4 kyrs BP	SST from C37 alkenones	Bard et al., 2000	−10.18	37.77	Portugal	H	N	Csa
Iberian Margin SST UK'37 17		0	0.4–31.4 kyrs BP	SST from alkenone unsaturation index UK'37	Bard et al., 2000	−10.18	37.77	Portugal	H	N	Csa
North Atlantic SST		0	0–110 kyrs BP	Measured with the alkenone method	Bard, 2003	−10.18	37.77	Portugal	H	N	Csa
18	Lake Ammersee T	500	0–15 kyrs BP	δ18O	von Grafenstein et al., 1999	11.00	48.00	Germany	H	N	Cfb
19	Lake Heimerdalsvatnet TIC	5	0–7.77 kyrs BP	TIC	Balascio et al., 2011 Balascio	13.39	68.18	Norway	H	N	Cfb
20	Lake Heimerdalsvatnet TOC	5	0–7.77 kyrs BP	TOC	et al., 2011 D'Andrea et al.,	13.39	68.18	Norway	H	N	Cfb
21	Kongressvatnet T	110	232–2008	Summer temperature rebuilt from alkenone saturation index	2012 Parisi et al., 2014	13.93	78.00	Svalbard	H	N	ET
22	Koszeg GP	300	1740–2010	Grapevine phonological stage at 23 April	Wittmeier et al., 2015	16.33	47.22	Hungary	H	N	Cfb
23	Jokelvatnet GM 24	156	12.5 kyrs BP	Glacier mass from titanium in lake sediments	McCormick et al., 2012	21.70	70.17	Norway	H	H	Dfc
Dead sea LL		−380	−0.94–3042 yr BP	Discretized lake level	Kvavadze and Connor, 2005	35.50	31.50	Israel	N	H	Bwh
25	Abkazia TL	>770	0–11,600 BP	Timberline	Messager et al., 2017	41.14	43.41	Georgia	H	L	Dfc
26	Nariani LOI	2050	4415–14,020 BP	LOI	McCormick et al., 2012	41.41	43.40	Georgia	H	L	Dfc
27	Lake Van OI	1648	0–3820 BP	Discretized oxygen isotopes		42.30	38.18	Turkey	L	H	Csa
28	Lake Van *Abies* PQ	1645	0–11.5 kyrs BP	*Abies* (pollen)	van Zeist and Woldring, 1978	43.00	38.30	Turkey	H	H	Csa
29	Lake Van *Acer* PQ	1575	0–11.5 kyrs BP	*Acer* (pollen)	van Zeist and Woldring, 1978	43.00	38.30	Turkey	H	H	Csa
30	Lake Van *Alnus* PQ	1645	0–11.5 kyrs BP	*Alnus* (pollen)	van Zeist and Woldring, 1978	43.00	38.30	Turkey	H	H	Csa
31	Lake Van *Thalictrum* PQ	1645	0–11.5 kyrs BP	*Thalictrum* (pollen)	van Zeist and Woldring, 1978	43.00	38.30	Turkey	H	H	Csa
32	Lake Van *Vitis* PQ	1645	0–11.5 kyrs BP	*Vitis* (pollen)	van Zeist and Woldring, 1978	43.00	38.30	Turkey	H	H	Csa
33	Caspian sea LL	−28	−600, +1875 CE	Discretized lake level	Klige and Myagkov, 1992	50.18	41.18	Azerbaijan	N	H	Bsk
34	Caspian sea LL	−28	−9000, +2000 CE	Discretized lake level	Klige and Myagkov, 1992	50.18	41.18	Azerbaijan	N	H	Bsk
35	Kinderlinskaya cave T 36	240	0–11,754 BP	δ18O	Baker et al., 2017 Ricketts	56.90	54.20	Russia	H	N	Dfb
Lake Issyk-Kul T		1600	1–8.5 ka BP	δ18O	et al., 2001 Kathayat et al.,	77.15	42.30	Kyrgyzstan	H	N	Bsk
37	Sahiya cave T	1378	3750 BCE–1950 CE	δ18O	2017 Eichler et al., 2009	77.52	30.34	India	H	L	Cfa
38	Belukha T	4062	1255–1975	Ice core δ18O	An et al., 2011 McCormick	86.34	49.48	Kazakhstan	H	N	Dfc
39	Lake Balikun LOI	1575	0–9 ka BP	Discretized LOI	et al., 2012 Aono and	92.48	43.40	China	H	H	Bwk
40	Wulan TR	3190	857–2003 CE	Tree rings	Kazui, 2008	98.24	37.12	China	H	H	Bsk
41	Kyoto CFD	300–500	800–2004	Cherry (*Prunus jamasakura* Sieber) flowering dates		135.75	35.02	Japan			Cfa

ELA = equilibrium-line altitude of a glacier.
GP = grapevine phenology.
GHD = grapevine harvest date.
CFD = cherry flowering date.
GM = glacier mass.
LL = lake level.
Lon,lat = longitude and latitude (degrees geographic coordinates) – reported only for punctual data.
LOI = loss on ignition.
PQ = pollen quantity.
rpr R = subjective judgment on representativity for precipitation (High, Medium, Low, Null).
rpr T = subjective judgment on representativity for air temperature (High, Medium, Low, Null). SOM = soil organic matter.
SST = sea surface temperature.
T = temperature.
TIC = total inorganic carbon.
TL = timberline.
TOC = total organic carbon.
TR = tree rings.
R = precipitation.
yrs = years.

analysis of each series are presented in the supporting material (figures from S1 to S10 for causal factor, figures from S11 to S46 for proxies).

Considering the longitudinal factor, a total number of 186 peaks was detected for West while 193 for East, with a distribution among the different time spans similar between the two groups (see supporting materials - Table S1), so we outline that both the areas show comparable behaviors.

Firstly, a frequency analysis of peaks for West and East was performed adopting 1000-year classes, applying the Student *t*-test as confidence analysis. Results in Table 5 show that, with a confidence >99%, classes 0–1000, 2000–3000, 4000–5000, 5000–6000 and 6000–7000 show frequencies of peaks higher for West than East.

This could means that the effect of the Atlantic Ocean is higher on West with an attenuation of signal towards East. However, these results must be considered with great caution due to the low number of peaks >1000 years.

The frequency of peaks in the range 0–1000 years was analyzed in detail, considering 50-year classes (Table 6), applying the Student *t*-test as confidence analysis. Results show that:

in the 20% of the cases, West shows higher frequency than East, with a 95% confidence for classes 0–50, 300–350 and 950–1000 and with a 99% confidence for class 550–600. In these cases, it could be hypothesized that the signals of the Oceanic oscillators AMO and NAO are dampened moving Eastward.
in the 50% of the cases, East shows higher frequency than West, with a 95% confidence for classes 200–250, 650–700, 750–800, 850–900 and a 99% confidence for classes 150–200, 400–450, 450–500, 700–750, 800–850 and 900–950.
the 10% of the time series (50–100 and 100–150) shows the same frequency in the East and West sides with a 99% confidence.
nothing can be stated for the remaining 20% of classes.

Fig. 1 shows the percentage of Eastern and Western proxy series with peaks in the 50 year classes, number of peaks detected is also provided.

Periodic peaks shared by a remarkable number of proxies are reported in Tables S3 and S4. About these common peaks it can be stated that the poor representativeness of shortest periodic peaks (Schwabe solar cycle of 11 years, 9–10 years AMO and 8–14 years NAO - of course we do not refer here to the main, multidecadal, AMO and NAO periods) is due to the fact that many of the series are not representative of such brief periodic peaks. We did not find any causal factor to explain the West maxima of 75, 117, 123, 841, 1165, 1868, 2890, 3528 years and the East ones of 77, 112, 120, 847, 1158, 1850, 2850, 3494 years. On the other hand, all the other peaks can be associated with one or more causal factors.

Focusing on grapevine, 7 proxies directly related to vine (Table 7) show that 68, 74, 97, 120, 152, 179, 318 and 388 years are present both in one or more occidental series and in the East series of the lake Van pollen. Unfortunately, short time periods are under the threshold of detectability of the lake Van series. Moreover, proxy peaks at 26 and 97 years are associated with the whole set of causal factors, peak 36 with NAO, AMO, ENSO, while 54 with NAO and AMO, 65 with AMO, 140 with Solar Activity. The peaks at 43, 74, 120 and 300 are not related to the selected causal factors.

Maps in Figs. 2 and 3 show the percentage of causal factor periods detected in the proxy series. It can be seen that peaks of NAO, AMO and solar activity imprint the proxy series of the whole area while the effect of the ENSO signal is limited to latitudes below 50°N which is coherent with the equatorial origin of the signal.

We can clearly observe that different proxies that are geographically close as the ones from Burgundy, the Alpine ones, the ones from the Iberian Border or those from Lake Van, answer differently to the same causal factor. This could be explained, considering that i) each proxy is more or less susceptible to a given forcing and ii) macroscale forcings act on target proxies through a bulk of factors working at intermediate scales (e.g.: mesoscale atmospheric circulation patterns, geomorphologic and hydrologic variables). These latter can in some cases mask the macroscale effects or vice versa, amplify them.

Furthermore, to test the robustness of the results presented in the four maps of Figs. 2 and 3, the correlation between the percentage of peaks detected in the proxy series and the number of peaks potentially detectable in the causal series has been verified. In all four cases the R^2 obtained are very low (NAO 0.0852, AMO 0.001, ENSO 0.0609, SA 0.0076), confirming that the results are not influenced by the different length of the proxy series used.

The check of positive peaks among causal factors and proxy series shows:

An average correspondence between NAO and proxy series of 71%, with a minimum value of 33% (proxy series 20 - Lake_Heimerdalsvatnet_TOC). The signal is well diffused throughout the whole area of investigation and particularly strong in the SOUTH, with 18.8% of the variance explained by latitude ($r = 0.43$, p-value 0.005016, result significant at $p < 0.001$). This indicates that the whole area is affected by NAO, but that South is more sensitive to fluctuations of the index (South answers positively to very strong level of the Indices, while North is regularly affected by the Westerlies even when the Index level is lower).

An average correspondence between AMO and proxy series of 65% with a minimum value of 0% (series 9 - Lake_Folgefonna ELA, two possible cases, both negative). The signal is well diffused and stronger in the South, but only the 8.58% of the variance is explained by latitude ($r = 0.29$, p-value 0.065882, not significant at $p < 0.05$).

An average correspondence between ENSO and proxy series of 57% with 6 series getting 0% correspondence and 4 series not comparable due to mismatch in the length of the causal series when compared to the proxy ones. The signal is well diffused, stronger in the South with 27.9% of the variance explained by latitude ($r = 0.53$, p-value 0.000779, result significant at $p < 0.001$).

An average correspondence between SA and proxy series of 66% with a minimum value of 2% (series 17 - North_Atlantic SST). The signal is well spread all over the area of investigation and no correlation with latitude was detected ($r = 0.12$, p-value 0.469379, result not significant at $p < 0.05$).

For all the four causal factor no correlation with longitude was detected.

Finally, a comparison among vine related proxies and other proxies time series is shown in Figs. 4 and 5. Yellow belts represent vine series peaks (±2 standard deviations). The peaks of the other proxies time series are represented by colored vertical bars. Fig. 4 is related to the time span 0–200 years while Fig. 5 to the 200–400 years range. The series in good agreement with grape proxies are Beluka, Issyk Kul, Swiss Cherry CFD, Morocco and Cadagno.

5. Discussion

Climate is an inherently chaotic system with a sensitive dependence on initial conditions. This strongly limits the adoption of the principle of causality. For this reason, the concept of causality adopted in our work should be read as co-variability within a network of climate-indices and proxies, in agreement with the approach of Wyatt and Curry (2013) who adopted the stadium wave model to rationalize the existence of the multidecadal climate signal - the Atlantic Multidecadal Oscillation (AMO) - that originates dynamically in the North Atlantic

Table 3
Periods (year) detected in the time-series of causal factors.

Time series	Periods
AMO	66.7, 34.8, 26.2, 10
AMO proxies	1506, 471, 235, 193, 99, 57, 34
NAO	382, 59, 36, 21, 16
NAO proxies	695, 347, 263, 201, 170
ENSO-Nilometer	242, 99.6, 82.6, 37.2, 23, 18.3
Solar activity SA	9370, 2205, 1442, 986, 721, 499, 351, 207, 140, 134, 87, 64, 22, 11
Mediterranean Oscillation Index MOI	30.6, 14.5, 3
East Atlantic Pattern EA	68, 30, 17, 9, 4
East Atlantic/West Russia Pattern EAWR	68, 30, 9, 6
Polar-Eurasia Pattern POL	68, 27, 14, 3
Scandinavia Pattern SCA	39, 17, 13, 9, 6
AMO_syn	1506, 471, 235, 193, 99, 66.7, 57, 34.4, 26.2, 10
NAO_syn	695, 382, 347, 263, 201, 170, 88, 59, 36, 21, 16

Table 4
Periods (year) detected in the analyzed time series. The time series referred to grapevine are in bold.

Time series	Periods maxima
Morocco TR	550, 241, 175, 96, 65, 48, 34
Medoc GHD	248, 103, 69, 41, 21
Lake Redon T	1391, 580, 331, 268, 178, 151, 124, 90.3, 74.8, 57.5, 33.9, 29.3, 26, 19, 16.6, 13.4
Burgundy C GHD	790, 316, 151, 99, 66, 52, 43, 37, 28
Burgundy D GHD	326, 155, 121, 76, 65, 57, 44, 36, 28
Burgundy T GHD	330, 150, 103, 75, 53, 46, 35, 27
Beaune GHD	399, 152, 118, 91, 73, 64, 44, 36, 24
Lake Verney LOI	5100, 3500, 2350, 1700, 1400, 590, 390
Lake Folgefonna ELA	3310, 1850, 1200, 950, 580, 270
Lake Bachalpsee LOI	5200, 3000, 2300, 1950, 1640, 672, 254, 231, 157, 142, 114, 107, 8.7, 78, 67
Swiss Plateau CFD	364, 145, 91, 47, 27
Swiss plateau GHD	382, 119, 91, 71, 55, 40, 23
Lake Cadagno T	5500, 3200, 2400, 1890, 677, 340, 234, 201, 173, 155, 142, 137, 117
Tirano GHD	170, 64, 38, 32, 26, 20
Iberian Margin SST C37	4100, 750, 610, 210, 174, 154, 143
Iberian Margin SST UK'37	3100, 590, 420, 320, 250, 240, 148, 125, 119
North Atlantic SST	12,500, 4400, 1100, 340, 250
Lake Ammersee T	6200, 3000, 3900, 1480, 1360, 960, 770, 725, 643, 529, 318, 293
Lake Heimerdalsvatnet TIC	3240, 2290, 1180, 750, 500, 320
Lake Heimerdalsvatnet TOC	7780, 6480, 2780, 1560, 1180, 864, 377
Kongressvatnet T	1780, 888, 555, 296, 193, 125
Koszeg GP	330, 102, 63, 47, 13
Jokelvatnet GM	3900, 2700, 1900, 1600, 580, 340, 152
Dead sea LL	1206, 603, 448, 334, 280, 227, 183, 135, 120, 65
Abkazia TL	6400, 2100, 1600, 1100, 890, 570, 120, 105, 95, 85, 50
Nariani LOI	9600, 4000, 3000, 1200, 800, 480, 350, 203, 189, 174
Lake Van OI	915, 696, 500, 311, 183, 120, 68, 58, 43
Lake Van *Abies* PQ	11,000, 3660, 1080, 946, 742, 335, 191, 165, 149, 133, 118
Lake Van *Acer* PQ	9400, 3100, 1560, 1170, 813, 650, 355, 208, 223, 191, 158, 148, 130, 106
Lake Van *Alnus* PQ	14,000, 3500, 950, 790, 431, 267, 208, 189, 167, 142, 110, 119, 114, 106
Lake Van *Thalictrum* PQ	8000, 1400, 1200, 675, 539, 449, 310, 191, 169, 141, 111
Lake Van *Vitis* PQ	7000, 3300, 1300, 1100, 850, 560, 450, 384, 300, 221, 170, 151, 144, 120, 102, 68
Caspian sea LL	7800, 3600, 2400, 1900, 1470, 870, 730, 470
Caspian sea LL	1235, 770, 618, 458, 386, 263, 199, 167, 131, 95, 85, 49, 34
Kinderlinskaya cave T	620, 178, 73, 55
Lake Issyk-Kul T	5400, 2700, 1400, 1100, 859, 394, 298, 203, 186, 167, 154, 142, 137, 120, 106
Sahiya cave T	779, 670, 465, 327, 253, 229, 215, 134, 77.9
Belukha T	584, 243, 172, 97, 75, 49.5
Lake Balikun LOI	7200, 3300, 2400, 1800, 1500, 720, 267, 153, 131, 114, 104, 100, 79, 68
Wulan TR	382, 164, 109, 79, 66, 59, 49, 32
Kyoto CFD	1193, 542, 271, 175, 97.8, 69.4, 38.2, 22.1, 16.7, 14.5, 11.4

Ocean and propagates throughout the Northern Hemisphere via a bulk of atmospheric and oceanic processes.

Many authors (see for example Mariani and Zavatti, 2017; Yoo et al., 2013) highlighted that a strong imprinting to mid latitudes climate is given by short-term variability (cycles with periods below 10 years), driven by causal factors like ENSO, NAO and Quasi Biennial Oscillation – QBO. In this work, periods below 10 years were not considered, presupposing that viticulture is able to thrive in a given territory only adapting to this kind of variability. On the other hand, cycles with periods from decades to millennia are crucial for the survival of viticulture. Marginal areas where viticulture is more subject to climatic risk are the most affected by these cycles that could lead to the abandonment of viticultural activities.

The discussion will follow the above-described causal chain.

A wide corpus of works based on archaeological evidence and documents describes the millennial cycles that affected the Holocene with the alternation of climatic optima and deteriorations (Lamb, 1966; Behringer, 2009).

More specifically, the solar cycles of Bray (2310 ± 300 years) and Eddy (967 ± 53 years) are reflected in the cycles of atmospheric and oceanic forcings and grapevine proxies analyzed in this work (McCracken et al., 2013).

A signature of millennial cycles is also observed in grapevine domestication and subsequent migration. Domestication took place during the great postglacial optimum (8000–5000 BP) (Terral et al., 2010) while the spread of grapevine to Western Europe and North Africa occurred from 5000 BP with the arrival in Italy recorded during the Mycenaean optimum (3500–3000 BP) (Terral et al., 2010). A push to colonize the rainy Oceanic environments was recorded during the Roman optimum (2300–1600 BP) when viticulture spread to Burgundy, Loire, Normandy, England, Rhine and Mosel areas (Brown et al., 2001). During the deterioration of the High Middle Age (1600–1100 BP) grapevine disappeared from the European oceanic environments, concurrently with the fall of the Roman Empire, which had formerly guaranteed the stability necessary for the affirmation of a perennial crop like vine.

A new expansion of viticulture was recorded during the MCO (1200–700 BP) when viticulture was practiced in North Germany and England (Lamb, 1966) while a new retreat took place during the LIA (700–150 BP), followed again by the current new progress.

In spite of the negative effect of climate deteriorations on the extent of viticultural areas, during the iron age deterioration (2700–2300 BP) viticulture reached South France, with the foundation of Marseilles by the Greek Phocaeans, and Spain with the arrival of Phoenicians or Greeks.

The Eastward expansion of viticulture is much less documented and also influenced by the different habits of the civilizations that came in contact with viticulture. During the development of this work we did not found time series of viticultural proxy data for the Eastern part of the study area. A possible source of information could be given by long time series of pollen of *Vitis vinifera*. Currently, we were able to use only the pollen time series from Lake Van, which led to interesting evidences (agreement with Western grape series).

Well documented by many authors are centennial and decadal cycles induced by SA (Chen and Zhou, 2012; McCracken et al., 2013), AMO (Wyatt et al., 2011; He and Wang, 2013) NAO (Mariani and Zavatti, 2017; Luo et al., 2016a, 2016b) and ENSO (He, 2015). These cycles were also detected in the forcings and viticultural proxies analyzed

Table 5
Analysis of occurrences of the periodic maxima for West and East for classes of 10,000 years.

Classes	Total available series	Available series		Positive series		Positive series (%)		Total number of peaks	Number of peaks		Tested hypotheses for percentages		Final evaluation
		West	East	West	East	% West	% East		West	East	H0: West = East	H1: West ≠ East	
0–1000	41	23	18	23	18	100.0	100.0	297	145	152	1	0	West = East
1000–2000	29	13	16	11	12	84.6	75.0	36	17	19	0.281755	0.718245	NE
2000–3000	24	11	13	7	5	63.6	38.5	13	8	5	6.86E−03	0.993143[c]	West > East
3000–4000	22	11	11	7	7	63.6	63.6	14	7	7	1	0	West = East
4000–5000	19	8	11	2	0	25.0	0.0	2	2	0	1.88E−04	0.999812[c]	West > East
5000–6000	17	7	10	3	1	42.9	10.0	4	3	1	1.68E−03	0.998321[c]	West > East
6000–7000	13	4	9	2	1	50.0	11.1	4	2	2	9.78E−03	0.990221[c]	West > East
7000–8000	11	3	8	1	4	33.3	50.0	4	1	3	0.386903	0.613097	NE
8000–9000	9	3	6	0	0	0.0	0.0	0	0	0	NA	NA	NE
9000–10,000	8	2	6	0	2	0.0	33.3	2	0	2	0.106558	0.893442	NE
10,000–11,000	5	2	3	0	1	0.0	33.3	1	0	1	0.272228	0.727772	NE
11,000–12,000	2	1	1	0	0	0.0	0.0	0	0	0	0.281755	0.718245	NE
12,000–13,000	2	1	1	1	0	100.0	0.0	1	1	0	NA	NA	NE
13,000–14,000	2	1	1	0	1	0.0	100.0	1	0	1	NA	NA	NE
14,000–15,000	0	0	0	0	0	NE	NE	0	0	0	NA	NA	NE
	0	0	0	0	0	NE	NE	0	0	0	NA	NA	NE

NA = not applicable, NE = not evaluable.
[a] Is for confidence >90%.
[b] Is for confidence >95%.
[c] Is for confidence >99%.

in Table 7. In this regard, it is interesting to note that the 99-year cycle recognized for AMO and the 99.6 years of ENSO are compatible with the Pacific Centennial Oscillation predicted by Coupled GCMs as described by Karnauskas et al. (2012).

In this work the forcings were considered mutually independent, even if interactions between atmospheric and oceanic indices AMO and NAO were outlined by Knudsen et al. (2014), while the influence of SA on NAO and Pacific Decadal Oscillation – PDO – was described by van Loon and Meehl (2014), who also highlighted the influence of PDO on ENSO. Moreover, García-García and Ummenhofer (2015) documented the joint effect of AMO and ENSO seasonal amplitude of precipitation on continental areas of the whole globe, showing that ENSO acts as modulator of the effects of AMO. The ENSO interaction with monsoon and AMO is important for viticulture domestication and spread because there are increasing evidences of a dampening of ENSO during Early and Mid Holocene and recent Dryas (White et al., 2018). A possible extension of the work could concern the spectral analysis of the warm events affecting the equatorial East Atlantic area (Carton and Huang, 1994).

Working on the pollen record of the Nariani wetland (2058 m asl), Messager et al. (2017) observed that East showed a delay in the postglacial forest expansion which was attributed to the effect of the Black Sea lake (the sea was 200 m lower than today and cold because of water coming from the melting of the Central Europe ice cap, before the breaking of Bosphorus and the flood fed by warm salty sea water), possibly strengthened by an analogous effect in the Caspian sea area.

On the other hand Roberts et al. (2012), working on high-resolution palaeolimnological data from Northern Spain, highlighted the prevalence of dry conditions during MCO caused by a persistent positive NAO state (Trouet et al., 2009) and a transition to more humid

Table 6
Analysis of occurrences of the periodic maxima for West and East for classes of 50 years.

Classes	Total available series	Available series		Positive series		Positive series (%)		Total number of peaks	Number of peaks		Tested hypotheses for percentages		Final evaluation
		West	East	West	East	% West	% East		West	East	H0: West = East	H1: West ≠ East	
0–50	18	10	8	10	5	100.0	62.5	32	24	8	1.34E−02	0.986551[b]	West > East
50–100	21	11	10	11	10	100.0	100.0	44	22	22	1	0	West = East
100–150	31	15	16	12	13	80.0	81.3	46	18	28	1	0	West = East
150–200	35	18	17	11	13	61.1	76.5	35	14	21	7.29E−05	0.999927[c]	West < East
200–250	34	17	17	6	8	35.3	47.1	16	7	9	1.14E−02	0.988555[b]	West < East
250–300	36	19	17	7	7	36.8	41.2	14	7	7	0.200284	0.799716	NE
300–350	36	19	17	10	7	52.6	41.2	17	10	7	1.36E−02	0.986396[b]	West > East
350–400	35	18	17	5	5	27.8	29.4	10	5	5	0.107245	0.892755	NE
400–450	33	16	17	1	3	6.3	17.6	4	1	3	5.63E−04	0.999437[c]	West < East
450–500	33	15	18	0	5	0.0	27.8	5	0	5	9.38E−10	1	West < East
500–550	33	15	18	2	3	13.3	16.7	5	2	3	0.325082	0.674918	NE
550–600	32	15	17	7	4	46.7	23.5	11	7	4	2.55E−06	0.999997[c]	West > East
600–650	30	14	16	2	3	14.3	18.8	5	2	3	0.254931	0.745069	NE
650–700	30	14	16	2	4	14.3	25.0	6	2	4	1.63E−02	0.983741[b]	West < East
700–750	30	14	16	1	3	7.1	18.8	4	1	3	1.99E−03	0.998014[c]	West < East
750–800	30	14	16	4	3	28.6	18.8	7	4	3	4.14E−02	0.958635[b]	West > East
800–850	29	13	16	0	2	0.0	12.5	3	0	3	2.45E−05	0.999976[c]	West < East
850–900	29	13	16	2	4	15.4	25.0	5	2	3	3.81E−02	0.961936[b]	West < East
900–950	29	13	16	0	3	0.0	18.8	3	0	3	2.45E−05	0.991489[c]	West < East
950–1000	29	13	16	2	1	15.4	6.3	3	2	1	1.34E−02	0.986551[b]	West > East

NA = not applicable, NE = not evaluable.
[a] Is for confidence >90%.
[b] Is for confidence >95%.
[c] Is for confidence >99%.

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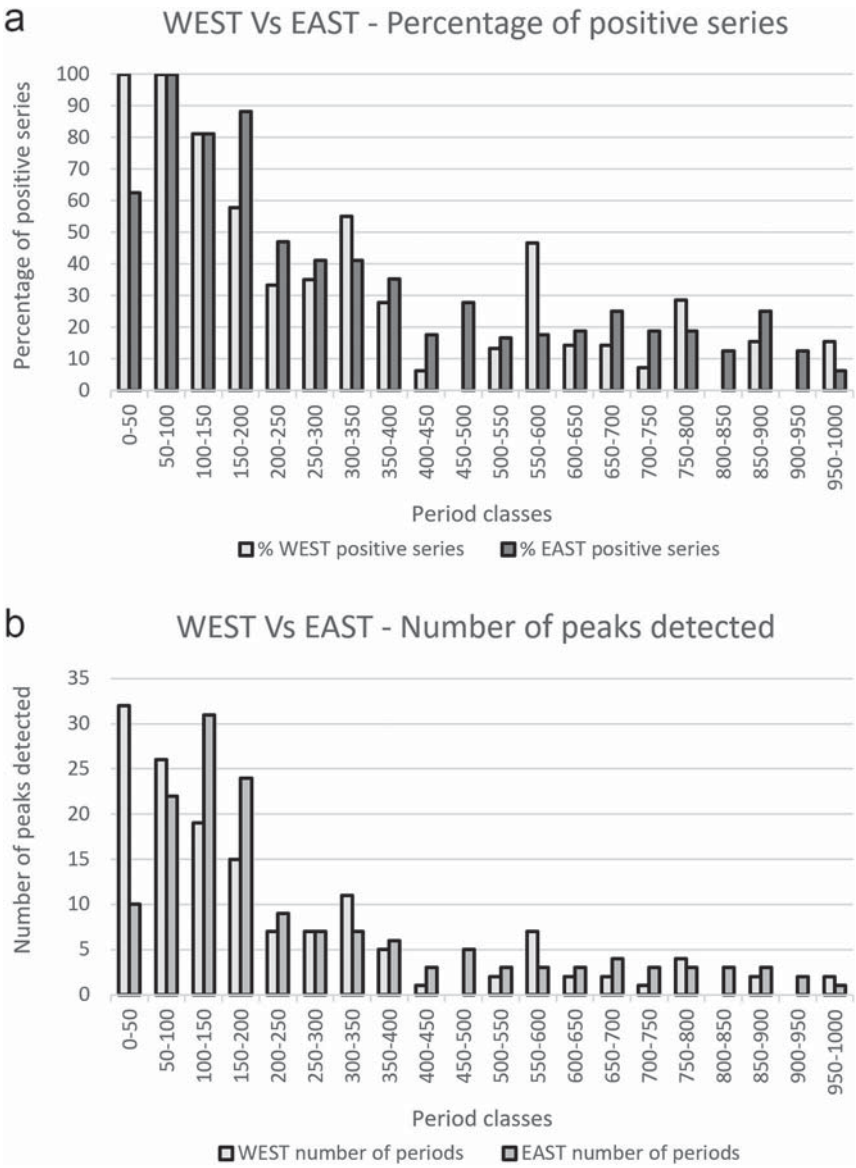

Fig. 1. Comparison between Eastern and Western proxy series: a) percentage of Eastern and Western series with peaks in the 50 year classes on the 0–1000 range, b) number of peaks detected for each 50 year class.

Table 7
Coherence of peaks detected in grapevine proxies and relations with peaks in causal factors (in bold the peaks common to more than one grapevine proxy).

		Peak number																					
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	***9***	***10***	*11*	*12*	***13***	*14*	***15***	***16***	***17***	*18*	*19*	*20*	*21*	*22*
Causal factors	**NAO**						695			382		201	170				88			59		36	21
	AMO			1506					471			235	193				99		66.7	57		34.4	26.2
	ENSO											242					99.6					37.2	23
	Solar Activity			1442	986		721		499	351		207			140		87						22
Grapevine proxies	Burgundy C GHD						790				316			151			99		66	52	43	37	28
	Burgundy T GHD										330			150			103	75		53	46	35	27
	Burgundy D GHD										326			155		121		76	65	57	44	36	28
	Beaune GHD									399				152		118	91	73	64		44	36	24
	Swiss plateau GHD									382						119	91	71		55	40		23
	Tirano GHD												170						64			38	26
	Lake Van pollen	7000	3300	1300	1100	850		560	450	384	300	221	170	151	144	120	102		68				
	Mean	7000	3300	1300	1100	850	790	560	450	388	318	221	170	152	144	120	97	74	65	54	43	36	26
	Standard deviation									9.3	13.4		0.0	1.9		1.3	5.8	2.2	1.7	2.2	2.2	1.1	2.1

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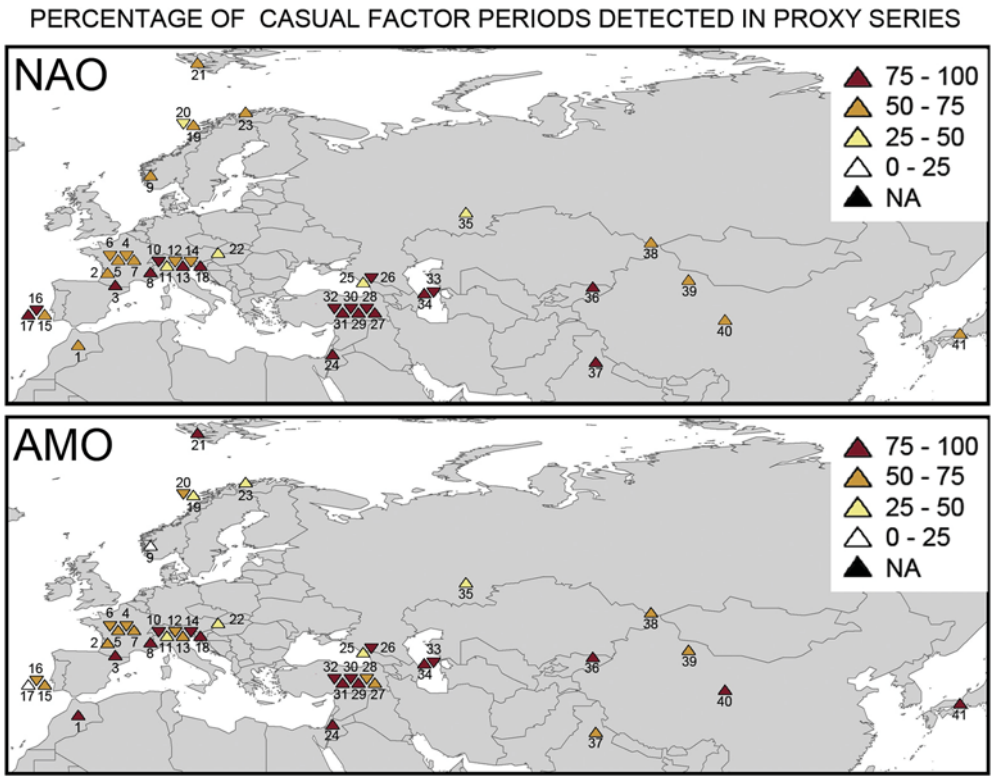

Fig. 2. Map of the coherence among proxy series and causal factors NAO and AMO.

conditions during the LIA. On the other hand, lake Nar (central Turkey) showed the opposite with a wet MCA and a dry LIA, as confirmed by the lake Van data oxygen isotopes (McCormick et al., 2012) and other marine and lake data from the Eastern Mediterranean area (Bakker et al., 2011). The conclusion of Roberts et al. (2012) is that an East–West bipolar climate see-saw operated in the Mediterranean for the last 1100 years. In other words, the see-saw mechanism could result in the 50% of West to East amplification of peaks frequency and in the 20% of West to East dampening of peaks frequency in the 0–1000 years range highlighted in our work.

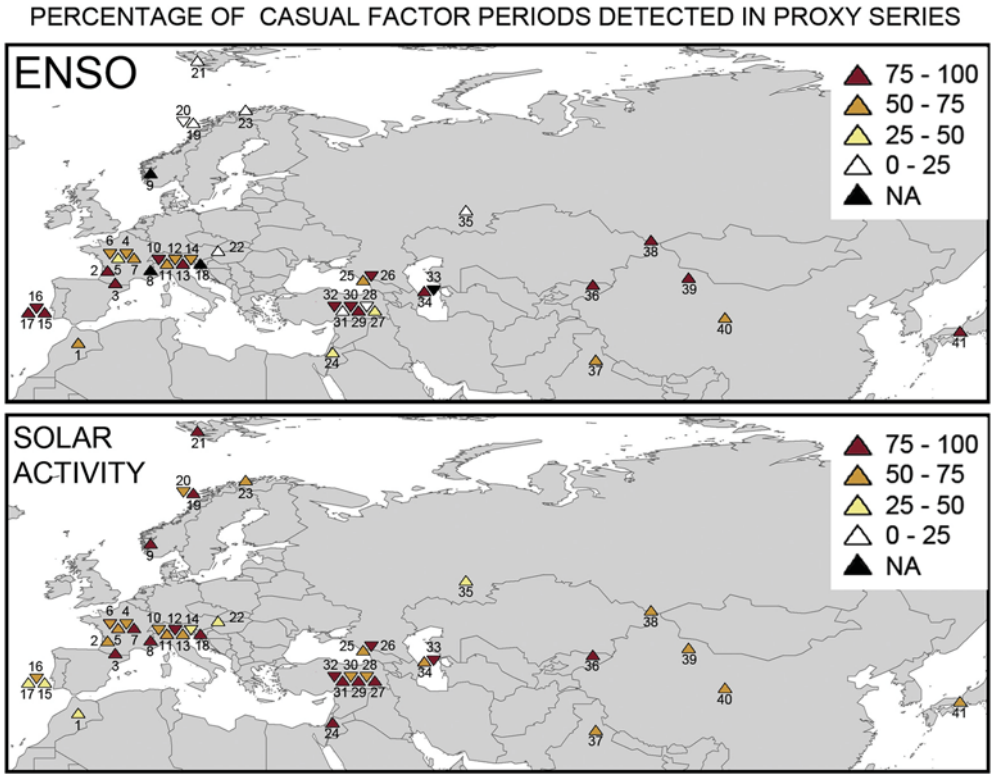

Fig. 3. Map of the coherence among proxy series and causal factors ENSO and Solar Activity.

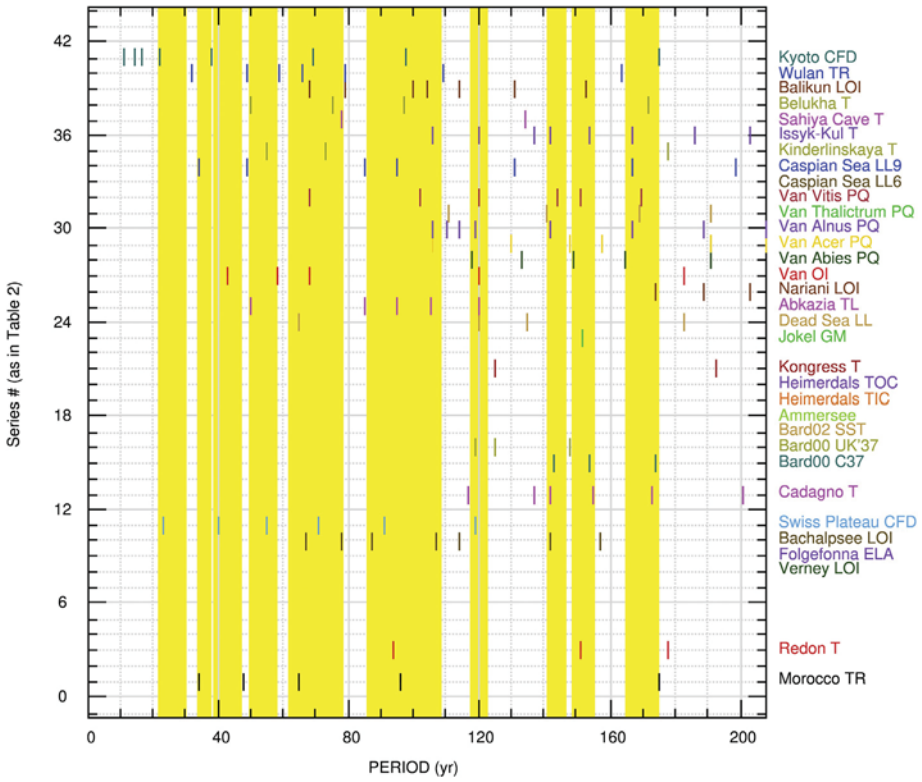

Fig. 4. Comparison of grapevine proxies peaks with other proxy series for the period range 0–200 years.

While dampening can be explained by the attenuation of the oceanic signal within a large continent (Driscoll and Yee Fong, 1992), amplification can be explained by physical mechanisms recently subject of relevant research works. Takaya and Nakamura (2005) highlighted that the winter amplification of the surface high over Siberia is associated with the formation of a blocking ridge in the upper troposphere over Central and Western Siberia, as a component of a quasi-stationary Rossby wave train propagating from the Atlantic across the Eurasian

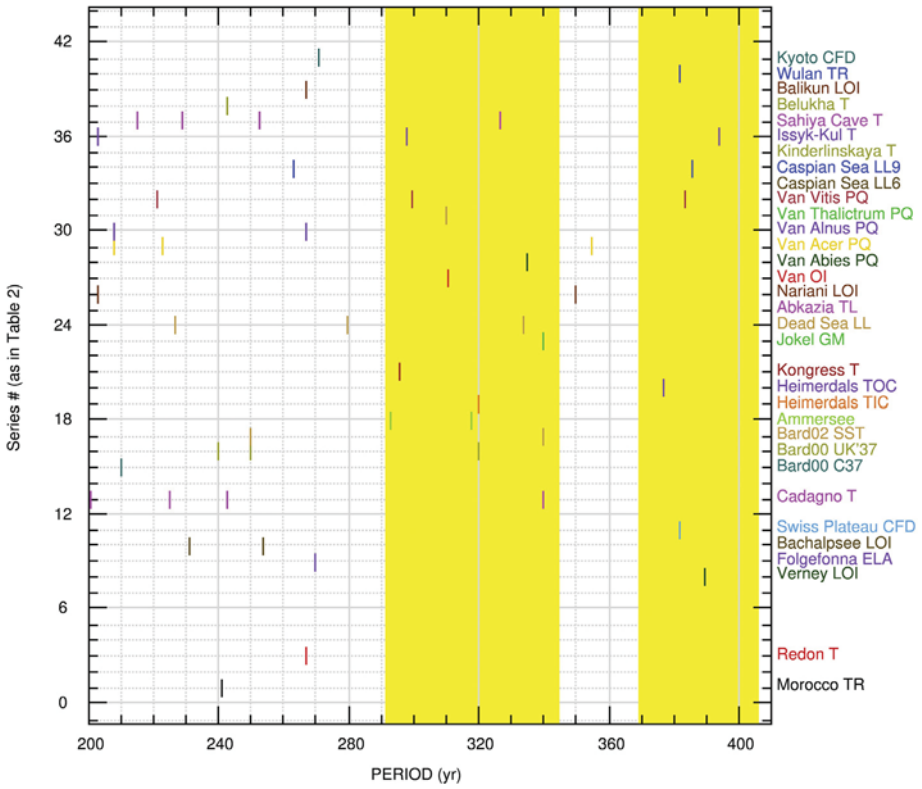

Fig. 5. Comparison of grapevine proxies peaks with other proxy series for the period range 200–400 years.

continent. Moreover Luo et al. (2016a, 2016b and 2017) showed a mechanism of Winter Eurasian cooling linked with NAO and AMO which affect the shape, frequency and persistence of Ural blocking (UB) events while Sun et al. (2015) highlighted the role of Atlantic Multidecadal Oscillation as a remote driver of Siberian Warm season precipitation, suggesting that the positive phase of AMO can excite an Eastward propagating wave train response across the entire Eurasian continent, including an East–West dipole structure over Siberia. This dipole then leads to anomalous Southerly winds, bringing moisture Northward to Siberia with a concurrent increase of precipitation. On the other hand, the amplification could be the expression of the structural instability of Eurasian climate under NAO forcing, shown by effects on air temperature and precipitation (Xu et al., 2016).

By a methodological point of view, it should be highlighted that one of the limits of the approach adopted in our work is given by the sources of uncertainty related to the proxy series, differing one of each other in terms of variable analyzed, time-length, period covered and so on. Furthermore, each series is subject to forcings active at different scales (macro, meso and micro) while our research focused only on macroscale factors.

As stated before a future development of this work could be given by the recovery of other historical series of biological relevance referring to cultivated plants and specifically to vines, such as, pollen or phenological series. The search of proxy series of different type, well related to grapevine ones, could also broaden the possibility of interpretation.

Another possible limitation is given by the presupposition that grapevine domestication and its successive spread from the center of domestication towards new areas was influenced only by climate while a very wide range of factors acted, including genetics, agronomy, commercial decisions and consumers likings.

With reference to genetics, the adaptation to new territories was probably favored by the presence of wild genotypes (*Vitis vinifera sylvestris*) capable of transferring genes to the domestic vine. *Vitis vinifera sylvestris* is a heliophilous trailing plant, which thrives in alluvial and colluvial woodlands, from the Himalayas to the Atlantic coast, between the 43th and 49th parallel (Bouby et al., 2013).

Regarding the technological influence of agronomy and commercial decisions, it should be recalled that the spread of the vine was favored by the easy transport of the propagation materials and by the technology of vineyard planting and managing. For example, the techniques described in the treaty *De re rustica* by Columella (e.g.: row cultivation, make of trenches named *pastinatio*, use of a tool named *ciconia* to verify the quality of the work done and so on) influenced the spread of viticulture in the wide territories of the Roman empire (Brown et al., 2001).

Moreover, the consumers liking played a relevant role in the success of viticulture as demonstrated by the end of the English viticulture, favored by the preferences given to Bordeaux wines (at that time under English domain) and by the consolidation of Northern France (Champagne) viticulture during LIA (Jackson, 2008) due to the commercial success of sparkling white wine.

6. Conclusions

Traditional viticultural terroirs of countries like Georgia, Spain, France and Italy are generally tolerant to the effects of climate cycles with period below 10 years, which are a relatively stationary component of climate risk. On the other hand, long cycles are more insidious because they are able to jeopardize the survival of viticulture with sequences of cold-rainy or hot-arid years that can discourage the vine growers. Moreover, the negative effects of these climatic cycles could have been more relevant in the past due to more limited technology.

In order to evaluate these aspects, the behavior of the different spectral peaks in 41 time series of geophysical and biological proxies, including 7 biological series for grapevine, were analyzed. The study was referred to a wide area ranging from the Iberian peninsula to Japan, also to highlight the effects of long-term variability on domestication and the spread of viticulture in Eurasia. The longitudinal and latitudinal behavior of signals was analyzed and related with some geophysical and solar causal factors acting at macroscale in our hemisphere.

The results obtained are relevant in terms of historical climatology, allowing us to identify how the climatic cycles had repercussions on the viticultural areas of Eurasia.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.04.175.

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Chapter 2

Archaiobotany

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ORIGINAL ARTICLE

Tracking the history of grapevine cultivation in Georgia by combining geometric morphometrics and ancient DNA

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Abstract

The Near East and the Caucasus are commonly regarded as the original domestication centres of *Vitis vinifera* (grapevine), and the region continues to be home to a high diversity of wild and cultivated grapevines, particularly within Georgia. The earliest chemical evidence for wine making was recorded in Georgian Neolithic sites (6000–5800 BC) and grape pips, possibly of the domesticated morphotype, have been reported from several sites of about the same period. We performed geometric morphometric and palaeogenomic investigations of grape pip samples in order to identify the appearance of domesticated grapevine and explore the changes in cultivated diversity in relation to modern varieties. We systematically investigated charred and uncharred grape pip samples from Georgian archaeological sites. Their chronology was thoroughly assessed by direct radiocarbon dating. More than 500 grape pips from 14 sites from the Middle Bronze Age to modern times were selected for geometric morphometric studies. The shapes of the ancient pips were compared to hundreds of modern wild individuals and cultivated varieties. Degraded DNA was isolated from three pips from two sites, converted to Illumina libraries, sequenced at approximately 10,000 single nucleotide polymorphism (SNP) sites, and compared to a large public database of grapevine diversity. The most ancient pip dates from the Middle Bronze Age (1900–1500 cal BC) and the domesticated morphotype is identified from ca. 1000 BC onwards. A great diversity of domesticated shapes was regularly seen in the samples. Most are close to modern cultivars from the Caucasian, southwest Asian and Balkan areas, which suggests that the modern local vine diversity is deeply rooted in early viticulture. DNA was successfully recovered from historic pips and genome-wide analyses found close parental relationships to modern Georgian cultivars.

Keywords *Vitis vinifera* · Domestication · Diversity · Caucasus · Outline analysis · Palaeogenomics

Introduction

Vitis vinifera ssp. *vinifera* (grapevine) was probably first domesticated in southwest Asia, where the most ancient archaeobotanical traces of grape cultivation have been found (for example, Zohary and Spiegel-Roy 1975; McGovern

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2003; Miller 2008; Fuller and Stevens 2019). At the same time, the genetic structure of modern cultivated grapes and relationships between cultivars and wild populations also support an origin of domesticated grape in the area from the Near East to Central Asia (Myles et al. 2011; Bacilieri et al. 2013; Emanuelli et al. 2013; Riaz et al. 2018), while the existence of secondary domestication events in the Mediterranean basin is still debated (Grassi et al. 2003; Arroyo-García et al. 2006). More specifically, the area south of the Caucasus, between the Black Sea and the Caspian Sea, was considered very early on by various scholars as the most likely place of origin of cultivated grape, due to the high local diversity of wild populations and cultivars (de Candolle 1886; Vavilov 1930; Negrul 1946; De Lorenzis et al. 2015). More than 500 grape varieties are considered to be native in Georgia (Maghradze et al. 2012). They comprise table and mostly wine varieties, with a large majority of white grapes. Among the most famous cultivars, 'Rkatsiteli', 'Saperavi' and 'Chinuri' are cultivated in eastern Georgia, 'Tsolikouri' and 'Tsitska' in the west. Genetic investigations confirm the specificity of the Black Sea-Caucasus germplasm compared to cultivars from other regions (Imazio et al. 2013; Liang et al. 2019) and a specific subcluster including most of the Georgian wine cultivars can be identified (Bacilieri et al. 2013; Laucou et al. 2018).

The high diversity of Georgian germplasm known today is probably a consequence of (1) the heterogeneity of environmental conditions, from the subtropical and Mediterranean climates close to the Black Sea to continental and mountainous ones in the north-east of the country, (2) the geographical location of Georgia, at the crossroads of north-south and east-west trade routes, and (3) the ancient and intensive wine growing tradition in the country. Homemade wine is still produced by most of the families in the countryside and local varieties cover 95% of the total area of vineyards in Georgia (Maghradze et al. 2012). Similar to the ancient Mediterranean practice, the typical Georgian tradition is to make wine in large pottery vessels set in the ground or buried, called *kvevri*, where the must ferments and wine is then stored (Beridze 1962; Reigniez 2016; Vigentini et al. 2016). Traditionally, grapes were simply pressed by foot in wooden containers and the resulting must was macerated in *kvevris* with a variable amount of skins, rachises and pedicels and for variable durations, depending on the type of wine that was to be produced. Due to its specificity and cultural significance, *kvevri* wine tradition was recently assigned the status of National Monument of Intangible Cultural Heritage by Unesco (https://ich.unesco.org/en/RL/ancient-georgian-traditional-qvevri-wine-making-method-00870). Based on linguistic, historical and archaeological data, the tradition of winemaking is thought to be deeply rooted in the history of Georgia (McGovern 2003; Maghradze et al. 2012). *Kvevri*-like storage jars are commonly reported from archaeological sites in Georgia. It has been supposed that similar moderately-sized jars already existing in the Kura-Arax culture (ca. 3500–1500 cal BC) and even in the Neolithic Shulaveri-Shomutepe culture (SSC; ca. 6000–4000 cal BC) could have been used to make and store wine (McGovern 2003; Batiuk 2013). This hypothesis recently received crucial support when chemical analyses of residues absorbed in pottery vessels showed evidence of winemaking at the SSC sites of Shulaveris Gora and Gadachrili Gora (5900–5500 cal BC) (McGovern et al. 2017). This result predates by at least 500 years the previous earliest evidence for wine, also obtained by chemical analysis, from the Neolithic site of Hajji Firuz Tepe (ca. 5400–5000 cal BC), in the northwestern Zagros mountains of Iran (McGovern et al. 1996), about 500 km south of Shulaveris and Gadachrili in Georgia. Both agree in identifying the wide area south of the Caucasus as the primary zone of the emergence of winemaking.

On the other hand, archaeobotanical evidence has been repeatedly invoked to defend the hypothesis of early viticulture in the southern Caucasus, starting from the 6th millennium BC. Sporadic finds of grape pips were reported from several Neolithic SSC archaeological sites in Georgia, Shulaveris Gora, Khramis Didi Gora, Dangreuli Gora (Gorgidze and Rusishvili 1984; Ramishvili 2001; Costantini et al. 2006; Rusishvili 2010). Additionally, grape pips were mentioned from Neolithic Chokh in the Russian province of Dagestan, in Aratashen, Aknashen and Masi Blur in Armenia, and from Shomu-tepe in Azerbaijan (Lisitsina and Prishchepenko 1977; Lisitsina 1984; Hovsepyan 2015). The morphology of the pips from some of these Neolithic sites, especially those from Shulaveris Gora, was regarded as typical of modern cultivated grapes, so grapevine would already have been domesticated by the 6th millennium cal BC in Georgia (Costantini et al. 2006).

The archaeobotanical documentation on the early history of viticulture in the southern Caucasus can however be considered as still limited and poorly known, first because of the small number of systematic archaeobotanical investigations there and because of the restricted literature available to international readers (Costantini et al. 2006). Taphonomic issues are not fully taken into account in the publications mentioning the finds of grape pips. It is sometimes difficult to understand if the pips were preserved by charring or another process. In many cases the excavations and original archaeobotanical studies were carried out many years ago and detailed information on the archaeological contexts and on sample composition is not always available.

In the framework of a national Georgian research programme (Maghradze et al. 2016) it was decided to systematically review the archaeobotanical grape pips, to obtain direct radiocarbon dates from them and to apply geometric morphometric (GMM) (Terral et al. 2010; Pagnoux et al. 2015) and palaeogenomic investigations (Ramos-Madrigal et al.

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Table 1 Investigated samples and radiocarbon dating results

Site name	Code	Location	Longitude	Latitude	Province	No of pips	Preserv.	Expected chronology	Lab ID	C %	$\delta^{13}C$ (AMS ‰)	^{14}C age, 1σ (yrs BP)	Calibrated age 2σ range	Cultural phase
Samples included in the study														
Pichori	PIC	Pichori	41.564626	42.450225	Samegrelo	1	Charred	2000-1500 BC	RTD 9042	52.0	-26.2	3,546±25	1940-1880 BC (58.5%) 1840-1830 BC (6.6%)	MBA
Dicha Gudzuba, Anaklia	DIC	Anaklia	41.58142	42.400642	Samegrelo	3	Uncharred	2000-1500 BC	RTD 7694	40.6	-28.4	3,369±37	1746-1604 BC (85.2%) 1588-1534 BC (10.2%)	MBA
Dedoplis Gora	DED1	Qareli District	43.71819	42.036598	Shida Kartli	52	Charred	1300-1000 BC	RTD-8892	68.8	-23.9	2,860±21	1110-975 BC (90.6%) 957-940 BC (4.8%)	LBA
Gabashvili Dateshidzeebis Gora	GAB	Kutaisi	42.72228	42.269335	Imereti	7	Uncharred	1000-700 BC	RTD-8900	58.7	-26.3	2,721±21	910-820 BC (95.4%)	IA
Uplistsikhe	UPL	Uplistsikhe	44.204167	41.968333	Shida Kartli	1	Charred	1000-900 BC						IA
Badaani	BAD	Tianeti District	44.966889	42.11	Mtskheta-Mtianeti	2 frg	Charred	3000-2000 BC	RTD 7640	65.7	-21.8	2,520±23	789-732 BC (26.2%) 691-661 BC (18.2%) 651-544 BC (51.0%)	IA
Digomi Room	DIGR	Tbilisi	44.783333	41.716667	Kvemo Kartli	49	Charred	1300-1000 BC	RTD 7637	70.2	-20.7	2,516±24	788-730 BC (24.3%) 692-660 BC (17.4%) 652-543 BC (53.7%)	IA
Arukhlo, Layer 4	ARU	Arukhlo	44.694444	41.466944	Kvemo Kartli	1	Charred	6000-5000 BC	RTD 7636	66.4	-21.6	2,445±22	750-687 BC (25.3%) 667-642 BC (7.6%) 593-409 BC (62.6%)	IA
Ergeta	ERG	Kolkheti Plain	41.673068	42.382353	Samegrelo	2	Uncharred	700-500 BC	RTD 7697	41.0	-26.9	2,445±34	753-685 BC (23.1%) 668-631 BC (9.6%) 626-611 BC (2.3%) 597-408 BC (60.4%)	IA
Treligorebi	TRE	Tbilisi	44.783333	41.716667	Tbilisi	33	Charred	800-600 BC	RTD-9425 RTD-9426 RTD-8899	64.7 65.6 77.0	-25.24 -27.21 -26.2	2,212±33 2,101±32 2,096±21	380-200 BC (95.4%) 335-330 BC (0.4%) 205-40 BC (95.0%) 180-50 BC (95.4%)	IA
Sukhumi	SUK	Sukhumi	41.022675	43.004445	Abkhazia	10	Uncharred	500-300 BC	RTD 7698	55.5	-27.4	2,118±33	347-320 BC (5.3%) 207-47 BC (90.1%)	IA
Tsikhia Gora, Kavtiskhevi	TSIK	Kaspi District	44.441542	41.81473	Shida Kartli	160	Charred	400-200 BC	RTD-7824	70.3	-24.9	2,107±20	193-86 BC (84.2%) 80-55 BC (11.2%)	Hellenistic
Dedoplis Gora	DED2	Qareli District	43.71819	42.036598	Shida Kartli	83	Charred	100-1 BC	RTD 7639	70.4	-20.4	1,960±29	40 BC-AD 87 (92.1%) AD 105-120 (3.3%)	Roman
Urbnisi cemetery	URB	Kareli District	43.977414	42.015984	Shida Kartli	1	Uncharred	AD 1-300						Roman
Lagodekhi	LAG	Lagodekhi	46.27614	41.820253	Kakheti	22	Uncharred	AD 1000-1400	RTD-7820	54.3	-27.3	423±16	AD 1436-1476	MA/Mo
Tsistamuri	TSIT	Tsistamuri	44.73285	41.86644	Mtskheta-Mtianeti	38	Uncharred	AD 1500-1700	RTD 7701	48.4	-20.6	426±31	AD 1422-1514 (89.8%) AD 1601-1617 (5.6%)	MA/Mo
Borjomi	BOR	Borjomi	43.35825	41.843901	Imereti	40	Uncharred	AD 1000-1300	RTD 7699	44.8	-19.8	247±27	AD 1525-1558 (7.5%) AD 1631-1678 (59.2%) AD 1765-1800 (23.9%) AD 1940-1955 (4.8%)	MA/Mo

Table 1 (continued)

Site name	Code	Location	Longitude	Latitude	Province	No of pips	Preserv.	Expected chronology	Lab ID	C %	$\delta^{13}C$ (AMS ‰)	^{14}C age, 1σ (yrs BP)	Calibrated age 2σ range	Cultural phase
Samples rejected														
Bichvinta - 23-T-1-4	BIC	Gagra District	40.42686	43.173697	Abkhazia	17	Uncharred	ca 21000 BC	RTD 7704	54.4	-21.3	99.9±1.3	Modern	
Gudou River, Section 3	GUD3		40.366052	43.212541	Abkhazia	55	Uncharred	7000-3000 BC	RTD 7702	55.6	-15.5	496±51	AD 1307-1363 (16.5%) AD 1385-1486 (78.9%)	
Gudou River, Section 5	GUD5	Gagra District	40.366052	43.212541	Abkhazia	80	Uncharred	7000-3000 BC	RTD-7703	29.0	-20.5	189±30	AD 1648-1694 (21.7%) AD 1727-1813 (52.8%) AD 1918-1955 (21.0%)	
Gadachrili Gora	GAD	Marneuli Plain	44.77	41.502883	Kvemo Kartli	13	Uncharred	6000-5000 BC	RTD 7600	58.7	-23.5	114.2±0.6	Modern	
Dangreuli Gora	DAN	Marneuli Plain	44.78	41.520104	Kvemo Kartli	5	Uncharred	6000-5000 BC	RTD 7647	50.0	-19.5	271±19	AD 1523-1572 (28.9%) AD 1630-1665 (65.1%) AD 1785-1794 (1.4%)	
Shulaveris Gora	SHU	Marneuli Plain	44.77	41.502883	Kvemo Kartli	8	Uncharred	6000-5000 BC	RTD 7648	52.7	-27.5	168±25	AD 1663-1697 (17.3%) AD 1726-1815 (53.3%) AD 1836-1878 (5.4%) AD 1916-1954 (19.4%)	
Samtavro	SAM		44.721278	41.847656,	Mtskheta-Mtianeti	50	Uncharred	1000-800 BC	RTD-7819	55.8	-23.4	167±15	AD 1666-1954	
Nastakisi	NAS		44.564464	41.864991	Shida Kartli	12	Uncharred	1000-500 BC	RTD 7696	43.7	-31.5	191±29	AD 1650-1691 (22.3%) AD 1728-1811 (53.8%) AD 1923-1955 (19.3%)	
Digomi Church	DIGC	Tbilisi	44.783333	41.716667	Kvemo Kartli	5	Uncharred	300-1 BC	RTD 7697	41.0	-26.9	117±25	AD 1680-1740 (27.3%) AD 1745-1750 (0.4%) AD 1750-1765 (2.2%) AD 1800-1900 (50.3%) AD 1900-1940 (14.6%) AD 1950-1955 (0.6%)	
Khizanaant Gora	KHI	Kareli District	43.958905	42.017095	Shida Kartli	60	Uncharred	AD 200-400	RTD 7705	53.1	-23.6	99.8±1.1	Modern	

All samples analysed for radiocarbon dating consisted of a single grape pip. The archaeological information and expected age are provided together with the chemical data. Carbon percentage (C%) is the carbon measured in the sample after pre-treatment. The stable carbon isotope ratio ($\delta^{13}C$) was measured with the accelerator mass spectrometer, so it does not represent the natural/charred isotope ratio

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2019) in order to (1) confirm the chronology of the findings, (2) identify when domesticated grapevine first occurred in the country and (3) explore how the cultivated grapes changed through time compared to the modern diversity.

Materials and methods

Vitis pip samples and radiocarbon dating

The *Vitis* pip samples available from archaeological archives and current archaeobotanical investigations were assessed and the related information on context and preservation conditions was recorded (Table 1). Carbonized grape pips have been recovered from eight sites and nine time periods, with an expected chronology according to the archaeological contexts ranging from the Neolithic to the Roman period (ca. 6000 cal BC–AD 500). Most of the samples, from 18 sites, were composed of uncharred remains, with an expected chronology ranging from the Palaeolithic until modern times and including several Neolithic sites.

Radiocarbon dating was done on 27 pips selected from 25 sites. Two samples, composed of isolated pips, could not be dated. The dating was carried out at the D-REAMS radiocarbon dating laboratory at Rehovot, Israel. Calibrated ages (95.4% probability) were obtained using OxCal v. 4.2 (Bronk Ramsey 2010) and the IntCal13 atmospheric curve (Reimer et al. 2013).

Geometric morphometrics (GMM)

Except the grape pip from Pichori, which could not be photographed before radiocarbon dating, and the sample from Badaani, which was only composed of broken pips, GMM investigations were performed on all the available samples (14 sites, 15 samples, 502 pips). Through the quantitative description of pip outlines using the Elliptic Fourier Transform method, GMM allows a powerful discrimination of wild and domesticated grape pips and characterization of the changes in cultivated diversity through time (Terral et al. 2010; Pagnoux et al. 2015). Each pip was photographed in dorsal and lateral views using an Olympus SZ-ET stereomicroscope and an Olympus DP12 digital camera. The images were converted to black silhouettes. The x and y coordinates of 360 equidistant points were sampled on each outline. Outlines were normalized before the EFT computations by centring, scaling using their centroid size and defining the first point right above the centroid. We used only the coefficients from the six first harmonics (48 coefficients) in the statistical analyses. All analyses were carried out using the Momocs outline analysis package (Bonhomme et al. 2014) in R environment (R Development Core Team, R v. 3.5.3.). Pip shape variation between archaeological samples was explored using principal component analysis (PCA) and linear discriminant analysis (LDA), performed on the 48 shape variables. In order to identify the wild or domesticated status of the pips and the closeness between domesticated archaeological pips and modern varieties we used predictive discriminant analysis. The archaeobotanical samples were compared to a reference collection of modern grape pips from 82 wild grapevines and 280 traditional cultivars considered as typical of various areas of Europe, the Mediterranean and the Caucasus (ESM 1, 2; Pagnoux et al. 2015). Wild grapes were sampled by us in several countries covering most of the distribution area of *V. vinifera* ssp. *sylvestris* in France, Germany, Georgia, Greece, Italy, Spain, Switzerland and Turkey. Most of our cultivars were selected and sampled from the INRA Grape Germplasm Repository, Domaine de Vassal, Marseillan-Plage, France, with the aim of being representative of the global diversity. Additionally, 43 native cultivars from Georgia were sampled from the Saguramo Grape Repository, Jighaura, Georgia. We have chosen wine and table varieties typical of various regions of Georgia.

The comparison of archaeological grape pips to the modern collection was carried out using two nested LDAs. We first compared the archaeological pips with modern wild (N = 2,430) and domesticated (N = 2,430) references. Then the domesticated-types were compared with modern varieties. When dealing with charred remains, we can assume that large assemblages are more likely to be well preserved compared to isolated pips, which should not be considered in cultivar level discriminant analyses (Bouby et al. 2018). In the present study, all the charred samples are composed of more than 30 pips and were therefore all considered in the cultivar level LDA. We consider the allocation by the LDAs reliable only when $p \geq 0.75$.

Ancient DNA

The preservation of ancient DNA (aDNA) in archaeological *Vitis* pips has been demonstrated by conventional methods of amplification and sequencing (Manen et al. 2003; Bacilieri et al. 2017). However, more robust analyses are made possible through high throughput sequencing, where millions of DNA molecules are sequenced in parallel. This approach has been shown to be useful on ancient grape pips (Wales et al. 2016), as it enables characterization of very short (< 50 bp) endogenous DNA molecules, including those with age-related chemical damage. Following a recently established methodology for *Vitis* archaeogenetics (Ramos-Madrigal et al. 2019), we performed such a shotgun sequencing and targeted enrichment of 10,000 single nucleotide polymorphism (SNP) loci in 17 grape pips from six archaeological sites, as summarized below.

DNA was extracted from archaeological pips in a dedicated aDNA facility at the University of Copenhagen, using

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a method developed for archaeobotanical remains (Wales et al. 2014), with modifications to retain ultrashort DNA (Dabney et al. 2013). The recovered DNA was converted to double-stranded DNA libraries using the NEBnext DNA Library Preparation Master Mix Set 2 (E6070L, New England BioLabs). The libraries were quantified using real-time polymerase chain reaction (PCR) to infer the appropriate number of PCR cycles needed to yield sufficient quantities of DNA for targeted enrichment experiments. Libraries were amplified with AmpliTaq Gold polymerase and sample specific indexes, and then screened for endogenous content on an Illumina HiSeq2500 sequencer. Samples with > 1% grape DNA were enriched for 10,000 informative SNP loci with a custom-designed MYbaits kit (Arbor Biosciences, Ann Arbor, MI, USA), following an established protocol (Ramos-Madrigal et al. 2019).

Processing of sequencing data was done following the approach described in Ramos-Madrigal et al. (2019). In brief, AdapterRemoval 2.0 was used to trim adapter sequences (Schubert et al. 2016), reads were mapped to the grape reference genome 12X.2 (Canaguier et al. 2017) using bwa aln (Li and Durbin 2009) and following aDNA standard practices, PCR duplicates were removed using picard tools, and reads with mapping qualities below 30 were excluded. The authenticity of the aDNA data was evaluated using bamdamage (ESM 3; Malaspinas et al. 2014). The archaeological samples were then compared to the GrapeReSeq modern reference database comprising 783 modern cultivars, 112 wild individuals and 11 other *Vitis* species (Laucou et al. 2018; Le Paslier et al. 2019). A principal components analysis (PCA) was performed using smart PCA lsq project (Patterson et al. 2012) including the samples in the GrapeReSeq database and the archaeological samples. To account for the low coverage of the aDNA data, we sampled a random allele for both of the archaeological samples and for each site in the reference panel before performing the PCA. Identity by state pairwise distances between archaeological samples and modern accessions were calculated using PLINK v. 1.9 (Chang et al. 2015) in order to identify the closest match between the archaeological samples and the reference cultivars. Finally, we used the genotype likelihood based approach implemented in NgsRelate (Korneliussen and Moltke 2015) to evaluate potential relatedness among the archaeological pips as described in Ramos-Madrigal et al. (2019).

Results

Authentication of archaeobotanical samples

Direct radiocarbon dating was crucial to validate the chronology of the archaeological pips. The age of many samples was confirmed by radiocarbon dating but several pips were found to be much more recent than the chronology expected according to the archaeological context (Table 1). Samples of charred plant remains were very little affected by these chronological readjustments. Many uncharred *Vitis* pips, on the other hand, were given a recent age. These should be regarded as contaminations of archaeological layers by modern intrusions and cannot be taken into account in our study. Such contaminations, relatively common in archaeological layers, were not always properly taken into account in archaeobotanical investigations in the past. Most of the samples that were rejected came from the eastern part of the country where the climatic conditions, drier than in the western part, are probably less favourable for the preservation of waterlogged plant remains.

After the validation procedure, the remaining dataset consisted of nine charred pip samples, originating from eight sites (N = 380), and eight uncharred pip samples from eight sites (N = 123) (Fig. 1). Intrusive pips especially affected the supposedly oldest samples, which were only composed of a few pips and dated to the Middle Bronze Age (1900–1500 cal BC). The Dedoplis Gora site provided a significant sample of charred material (NB = 52) dated to the Late Bronze Age (1110–940 cal BC).

The shape of the archaeological grape pips: wild and domesticated morphotypes

The first biplot of the PCA shows that the differences between the samples are weak compared to diversity between samples (Fig. 2). The unrooted neighbour joining tree realized after a LDA performed on the largest samples (N ≥ 20) nonetheless reveals a chronological trend in the organization of the samples. The existence of significant differences between the sites was checked beforehand by a multivariate analysis of variance MANOVA ($F(336, 2996) = 5.48$, $p < 0.001$) performed on shape descriptors and subsequent pairwise comparisons (ESM 4). It is noteworthy that the Late Bronze Age sample from Dedoplis Gora is the only one which does not fit this chronological organization, being closer to the Roman period sample from the same site than to Iron Age samples. This could potentially reflect a site effect, a local tradition, stronger than the larger scale chronological changes.

Preservation by charring usually causes some deformation of the pips. Experimental studies show, however, that this does not prevent geometric morphometric identification of wild and domesticated morphotypes, nor, for the well preserved samples, identification of modern varieties (Ucchesu et al. 2016; Bouby et al. 2018).

In the neighbour joining tree the uncharred pip samples are grouped together to form the entire medieval/modern period group and are separated from all the charred samples,

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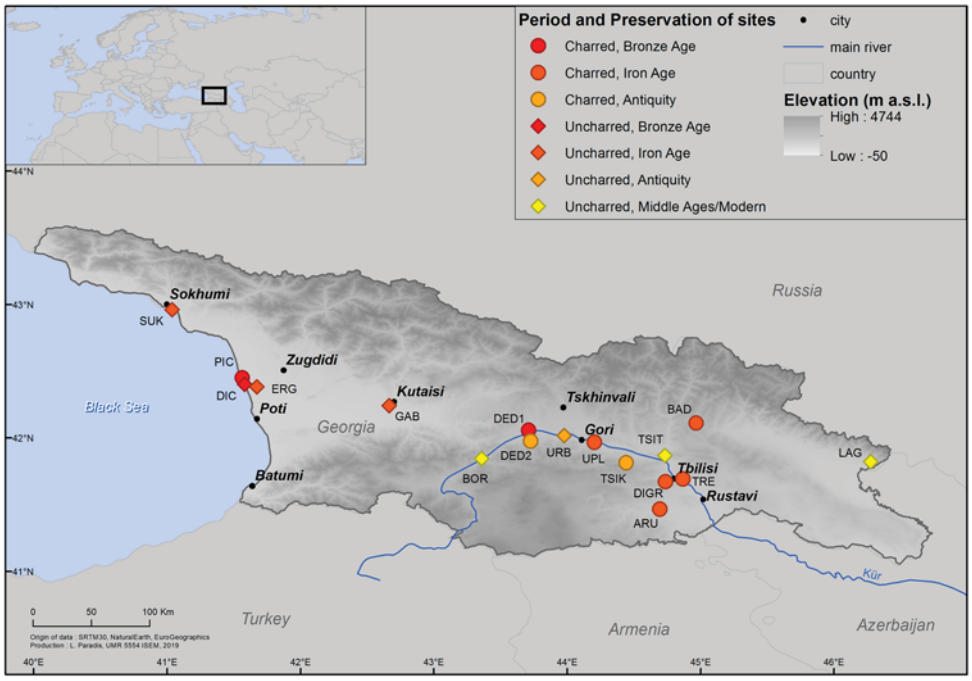

Fig. 1 Location map of the investigated sites; abbreviations for sites in Table 1

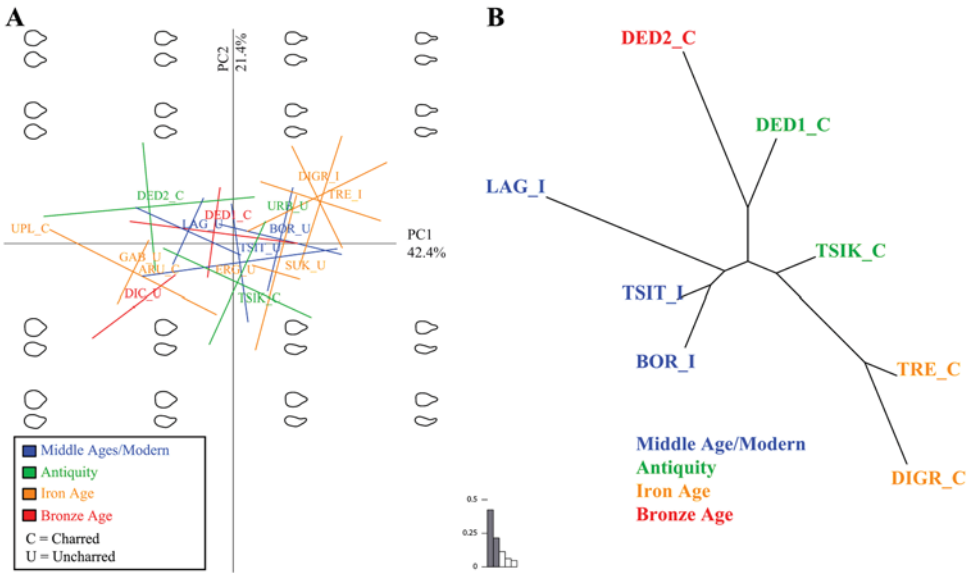

Fig. 2 Comparison of archaeological pip samples according to seed shape (48 EFT coefficients). **a** First biplot of the principal component analysis performed on all the samples; **b** Unrooted neighbour joining tree realized after a LDA performed on the largest samples (N ≥ 20)

all dating from earlier periods. It is therefore difficult to assess if this separation is partly caused by deformation due to charring or if it only reflects the general chronological trend.

Following Evin et al. (2015), leave-one-out cross-validation in a LDA performed on a balanced sample of domesticated and wild grape pips randomly selected from our original modern collection allows a very good classification of the pips into wild or domesticated status (95.7%). The classification in the LDA of the archaeological pips allows allocation of 51.8% of the pips to the domesticated-type morphotype and 28.3% to the wild-type morphotype (threshold $p \geq 0.75$; 19.9% non-allocated). The single Middle Bronze Age sample (from Dicha Gudzuba; 1746–1534 cal BC) is only composed of three pips allocated to the wild-type (Fig. 3, ESM 5). Later on, the domesticated-type is generally dominant in the samples. The most ancient occurrence is from the Late Bronze Age site of Dedoplis Gora (1110–940 cal BC), in the eastern part of the country (domesticated-type, 63.5%). In western Georgia, the oldest occurrence is from Iron Age Sukhumi (347–47 cal BC), but only a few grape pips were available from this region. It is of interest to note that the wild-type is well represented or dominant from the two Hellenistic and Roman sites. Later,

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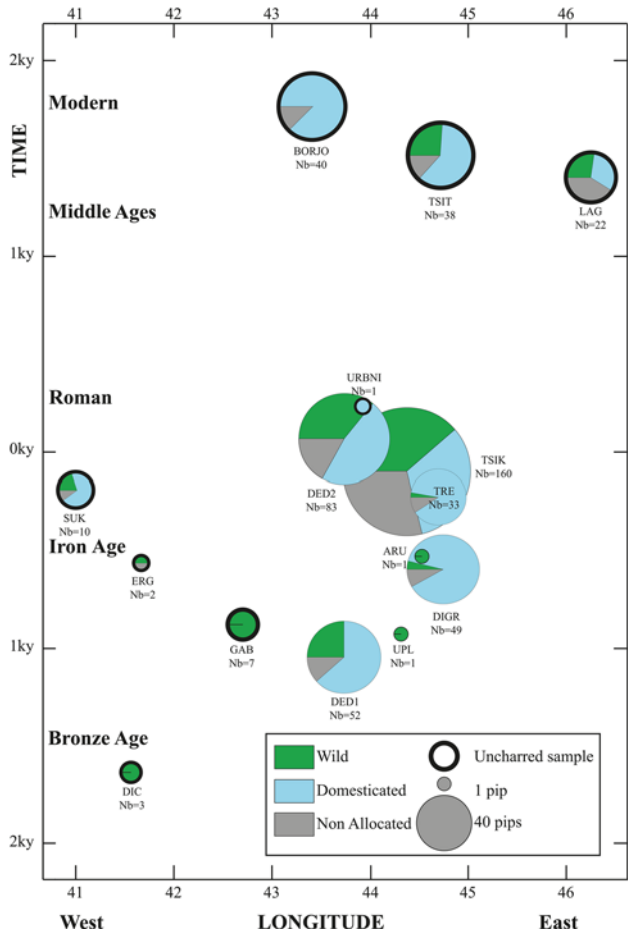

Fig. 3 Proportions of pips allocated by the LDA to the domesticated and wild morphotypes in each sample. The samples are arranged according to their chronology and location (longitude)

from the medieval and modern periods, its proportions seem to decrease.

Comparison of archaeological domesticated-type pips to modern varieties

The 269 pips allocated to the domesticated-type by the first LDA were then classified as additional individuals in a cultivar level second LDA, based on a modern collection of 280 varieties. Leave-one-out cross-validation allows a classification of 77.18% of the pips into the correct cultivar. This must be considered as a very high discrimination rate given the very large number of groups. From the 269 domesticated-type pips, 133 can be allocated to a specific modern cultivar with $p \geq 0.75$. This means that more than 50% of the domesticated-type archaeological pips cannot be attributed to our modern sample. They may correspond to cultivars not included in our comparison sample or to unknown or extinct forms.

The allocated pips match with 65 different modern cultivars (ESM 5). A high morphological diversity characterizes all the sites. Most cultivar types are not represented by more than one or two pips. The most common morphotypes match with 'Glycostaphyllo' (17 pips; 6 sites), 'Sliva' (8 pips; 4 sites), 'Qisi' (6 pips; 3 sites) and 'Jahafi' (5 pips; 4 sites). These morphotypes are not specific to any particular chronological period.

The identified morphotypes correspond to cultivars considered characteristic of different countries or large geographical areas (ESM 6). Forty of them are regarded as typical of the Caucasus, the Near East and the Balkans, particularly Greece. But some pips find their best match with cultivars considered as originating from other areas of Western Asia, North Africa and Europe, including several western European varieties. It should however be noted that the large majority of the pips is allocated to cultivars from the Caucasus, Near East and Balkans (Fig. 4). Moreover, when comparing the number of assigned pips to the number composing each geographical group in the modern collection, it is clear that the distribution of archaeological pips significantly differs from the modern sample ($Chi^2 = 12.584$, p value = 0.002, Fisher test p value = 0.001). The Caucasus and Near East group (EMCA) is over-represented with regard to the central and western European group (WCEUR). This pattern holds true regardless of the date of the samples (Fig. 5). During Antiquity (TSIK and DED2 sites) the proportion of pips whose shape is typical of cultivars originating from central and western Europe is higher. This however should be regarded very cautiously, as no significant difference can be detected between the chronological groups using a Fisher exact test (p value = 0.364).

Ancient DNA affinity to native Caucasian grape varieties

Shotgun sequencing revealed that a majority of the archaeological pips contained very low amounts of endogenous grape DNA (Table 2). Twelve pips yielded a percentage of reads mapping to the grape reference genome as low as the extraction control ($\leq 0.04\%$). Since the extraction control serves as a baseline to identify erroneous mapping of short DNA to the grape genome, as well as to monitor potential contamination, we concluded that the specimens from Treligorebi, Sukhumi, Dedoplis Gora and Lagodekhi provided no evidence for aDNA preservation. This finding was not unexpected for the charred pips from Treligorebi and Dedoplis Gora, as previous research has revealed that high throughput sequencing of charred plant remains rarely yields useful amounts of endogenous DNA (Nistelberger et al. 2016). However, given the rarity of uncharred pips from Georgia in this key chronological period, we dedicated the resources to fully explore the possibility of preserved DNA in them.

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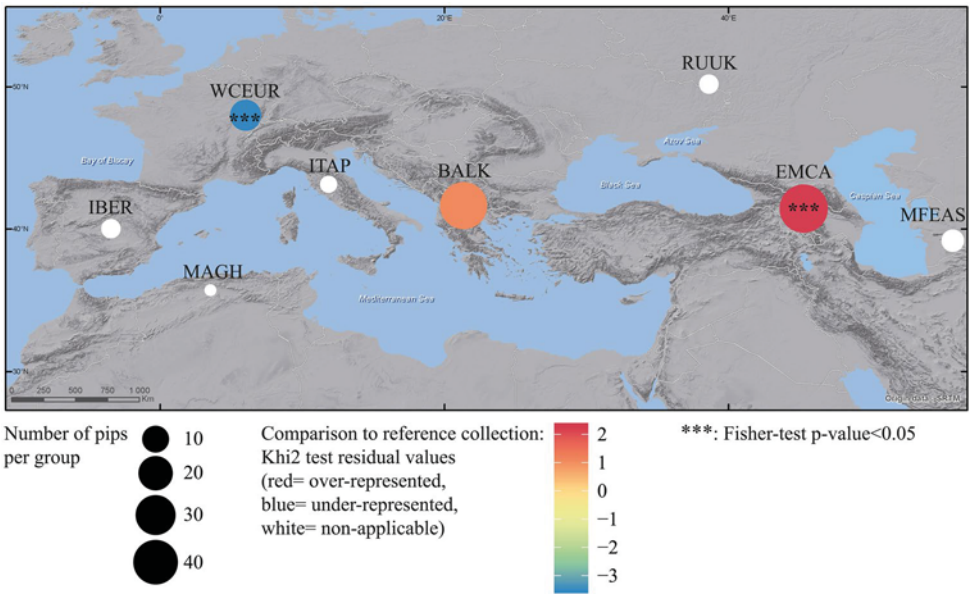

Fig. 4 Number of archaeological pips allocated to modern geographical groups and comparison of archaeological and modern distributions using Chi^2 (Khi^2) test; Chi^2 value = 12.584, DF = 2, p-value = 0.002. *IBER* Iberian Peninsula, *MAGH* northwest Africa, *ITAP* Italy, *RUUK* Russia; (others as for Fig. 5)

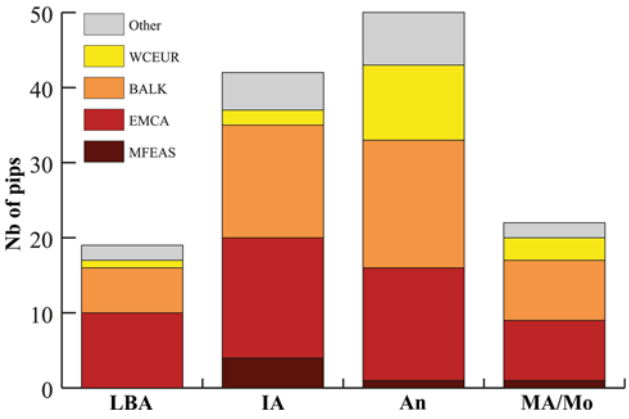

Fig. 5 Distribution of domesticated-type archaeological pips according to their date and to the geographical group of the identified cultivars. Periods, *LBA* Late Bronze Age, *IA* Iron Age, *An* Antiquity, *MA/Mo* Middle Ages/modern times; geographical groups, *MFEAS* Middle & Far East, *EMCA* Caucasus & Near East, *BALK* Balkans, *WCEUR* Western & Central Europe

The lack of endogenous DNA from the uncharred pips from Sukhumi and Lagodekhi suggests that other factors may have a significant impact on DNA preservation, such as microbial activity, age-related degradation, or specific soil chemistry.

Three pips from the most recent samples, two from Borjomi and one from Tsitsamuri, yielded > 1% endogenous DNA (1.89–10.74%) and were selected for in-solution targeted enrichment so they could be compared against the modern grapevine database. As is often observed by aDNA researchers (Carpenter et al. 2015), the fold enrichment on the targeted SNP loci was highly variable between samples, with moderate increases for the two Borjomi samples and high enrichment for the Tsitsamuri pip.

The three enriched samples produced low to medium coverage on the targeted SNP loci, which is sufficient for conducting broad ancestry assignment analysis and evaluating potential relatedness using genotype likelihoods given a reference panel with genotype data for modern cultivars (Ramos-Madrigal et al. 2019). Although fresh grape pips

Table 2 Archaeological grape pips analysed for aDNA and DNA preservation

Site	No of seeds	Preserv	Age	Reads mapping grape genome
Treligorebi	3	Char	380–50 BC	~0.02%
Sukhumi	5	Unch	347–7 BC	~0.01%; ~0.03%
Dedoplis Gora	2	Char	40 BC–AD 120	~0.01%
Lagodekhi	2	Unch	AD 1436–1476	~0.04%
Tsitsamuri	3	Unch	AD 1422–1617	**1.89%**; 0.58%; 0.54%
Borjomi	2	Unch	AD 1525–1955	**2.11%**; **10.74%**
Extraction blank	N/A	N/A	N/A	~0.04%

Bold values indicate the three pips that yielded more than 1% endogenous DNA and that were selected for targeted enrichment

contain a mixture of DNA from both parents, Ramos-Madrigal et al. (2019) demonstrated that archaeological pips are largely composed of maternal tissue, meaning that the genetic signature primarily originates from the plant carrying the grapes. A PCA including the archaeological samples and modern accessions in the GrapeReSeq database revealed that all three archaeological pips were most closely related to modern domesticated Georgian varieties (Fig. 6). Furthermore, when we estimate pairwise distances between the archaeological samples and the modern cultivars, the specimen with the highest coverage on the SNP loci, Tsitsamuri-3, was closest to 'Adreuli skelkana', a Georgian white grape variety (Maul et al. 2019). Finally, we estimated kinship coefficients between pairs of archaeological samples using NgsRelate and found that none of the pips showed patterns consistent with highly related samples (ESM 7).

Discussion

The beginnings of grape cultivation

It is difficult to establish the date when grapevine cultivation started in Georgia. The oldest grape pips dated with certainty go back to the Middle Bronze Age (1900–1500 cal BC) and belong to the wild morphotype. The domesticated morphotype is recorded and dominant in the samples only from the Late Bronze Age onwards (1110–940 cal BC). This most probably represents evidence of local vine growing, but it is very late compared to what was expected and to the very early chemical traces of wine from Shulaveris Gora and Gadachrili Gora, more than 4,500 years earlier. At these two sites, pottery jar base sherds sampled from layers dated to 5900–5750 cal BC and 5700–5500 cal BC revealed the presence of wine chemical biomarkers (McGovern et al. 2017). The statement that wine was contained in the jars was not based solely on the presence of tartaric acid, which can be judged inconclusive (Stern et al. 2008; Barnard et al. 2011), but on the joint identification of a variety of organic compounds thought to be typical of grapes and/or wine. Tartaric, citric and malic acids can be found in large amounts in dark grapes, while succinic acid is regarded as a fermentation marker (Garnier and Valamoti 2016). The combination of these different biomarkers is probably the strongest evidence for ancient wine that can be obtained through chemical analysis.

Based on the regional archaeological evidence, grapevine cultivation probably started in Georgia before the Late Bronze Age. If grape pip assemblages are more common and larger from this period, it is probably due to the intensification and spread of viticulture in the country then.

In the Near East to the south of the Caucasus, the most ancient evidence of grape cultivation possibly dates to the 5th millennium BC, when grape pips and pollen are recorded for the first time outside the natural range of wild grapevine (Fuller and Stevens 2019). But grape finds only become more widespread from the 4th millennium BC (Fuller and Stevens 2019) and the general cultivation of grapevine outside its natural range would have only occurred from the 3rd millennium BC (Miller 2008). By the 4th millennium BC grape pips are often found with fruit skins and pedicels in the sites of the Near East (Longford 2015). This suggests that grapes were not simply eaten but regularly used to make wine. In the Caucasus, a probable Chalcolithic wine making installation has been found in the cave complex of Areni-1, Armenia. It is composed of a basin-shaped clay platform draining into a large semi-underground jar, surrounded by numerous storage vessels (Areshian et al. 2012). Desiccated grape pips, fruit skins, rachises and pedicels were discovered nearby. Several

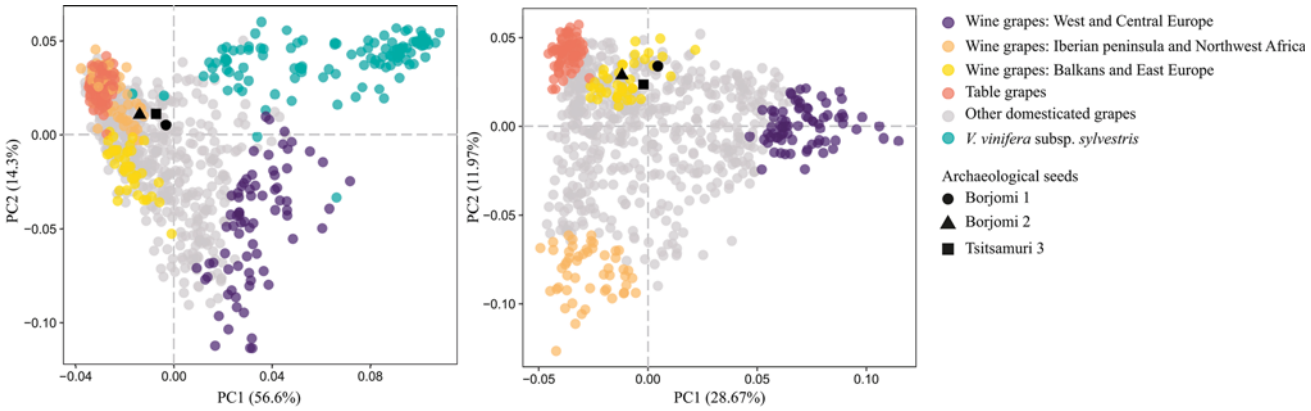

Fig. 6 Principal component analysis (PCA) biplot of archaeological pips and modern reference accessions from the GrapeReSeq database. Left, PCA including archaeological samples, wild grapevines and modern varieties; on right, PCA including only archaeological samples and modern varieties. For both the archaeological and GrapeReSeq samples a random allele was chosen for each genomic site in the database

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Vitis remains are dated from Late Chalcolithic times (ca. 4050–3800 BC), even if other *Vitis* remains are dated from the Bronze Age and medieval (Smith et al. 2014). The hypothesis of a grape pressing and wine-making installation is corroborated by chemical results showing the presence on potsherds of malvidin, an organic compound typical of red wine and pomegranate juice (Barnard et al. 2011). It is unknown if grapevines were already domesticated. No comprehensive research has been done on the morphology of grape pips from Armenia. The results obtained from the calculation of the Stummer Index (breadth/length) are inconclusive (Smith et al. 2014) and this index is in any case not very efficient when applied to modern pips (Bouby and Marinval 2001). Considering the regional context, grapevine was nevertheless probably cultivated in Areni about 4000 BC, therefore possibly also in neighbouring Georgia.

Wine from wild grapevines?

There is currently no archaeobotanical data to suggest that grapevine could have been domesticated as early as the beginning of the 6th millennium BC. An alternative hypothesis is that the first wine could have been made from wild grapes (Miller 2008). Microvinification experiments show that wild grapes are suitable for making wine fermented by wild yeasts, with a medium concentration of alcohol (ca. 11%) and a relatively high level of acidity (Arroyo-García et al. 2016). The main disadvantage of using wild vines is their smaller and irregular production of grapes.

Wild grapevine was probably already common when the first Neolithic inhabitants of the Shulaveri-Shomutepe culture (SSC) settled in Georgia. The area between the Black and Caspian seas is considered as the main Quaternary glacial refugium for grapevine (Naqinezhad et al. 2018). Scattered charred pips have been found at several Neolithic sites in the Caucasus area (McGovern et al. 2017). But as far as one can tell their morphology is of the wild-type. This is the case for three Neolithic and Chalcolithic (6th and 5th millennium BC) pips from Mentesh Tepe, Azerbaijan (Decaix and Bouby, unpubl.), where *Vitis* charcoal was also found, proving the local presence of the vine since the SSC (Decaix et al. 2016).

In Late Neolithic Dikili Tash, northern Greece, early wine making is suggested by the simultaneous presence of grape pressing residues (Valamoti 2015) and by chemical evidence of wine in associated vessels (Garnier and Valamoti 2016). The GMM study of these pips shows that only the wild morphotype was present (Valamoti et al. 2020) and therefore that this wine was produced from undomesticated grapes.

If these first Neolithic wines were produced from wild grapes, it is quite likely that the vines were cultivated or managed in order to improve and regularize their yield.

The diversity of cultivated grapevines

From the Late Bronze Age a large diversity of morphotypes of grape pips has been identified from the sites in Georgia. The wild morphotype is very common until the Middle Ages. It may represent grapes collected from wild individuals growing near the settlements. People in Georgia have been reported in the recent past to regularly make wine with grapes gathered from wild plants growing on trees in the mountains (Julien 1816), even if vines deliberately grown up trees, a common practice in the country until recent times, could have been occasionally confused with truly wild individuals.

On the other hand, the wild pip morphotype has been found repeatedly in many protohistoric and historic period sites in France and Greece, including vineyards and urban sites, leading to the hypothesis that it represented a cultivated form (Terral et al. 2010; Bouby et al. 2013; Pagnoux et al. 2015; Valamoti et al. 2020). This wild-type would then represent either truly wild individuals or plants that had already been selected for some desirable traits, but involving no identifiable change in pip morphology.

The morphology of the domesticated-type pips from Georgian sites is often close to that of modern grape cultivars typical of the Caucasus and southwest Asia. Many other pips are similar to modern varieties from the Balkans. The identified morphological resemblance cannot be considered as a direct identification of the cultivars. Our reference collection includes only a fraction of the thousands of described varieties. However, the morphological similarities probably express a relationship between the varieties cultivated today in the region and the vines cultivated there over the past 3,000 years. Genetic data show that most of the modern Georgian varieties are gathered into one specific small genetic group (Laucou et al. 2018). Microsatellite markers show that this group belongs to a bigger cluster mainly composed of table varieties from the eastern Mediterranean, western and Central Asia (Bacilieri et al. 2013). On the other hand, morphological resemblances have long been noted between Georgian grape varieties and wine varieties from Asia Minor and the Balkans. Negrul (1946) considered them as two sub-groups, sub-proles *balcanica* and *georgica* of his prole *pontica*. The predominant morphological proximities identified between ancient pips and modern cultivars from southwest Asia and the Balkans are therefore consistent with these relationships. Proximities identified with present varieties from other regions, such as the rest of Europe, may be explained by (1) the fact that not all western Asian are in our collection, ((2) morphological variability within modern

varieties or (3) deformation of some archaeological pips. Many of the pips allocated to European varieties are charred. Moreover, many modern cultivars are hybridised and cannot be assigned to any genetic group, probably as the result of long-distance exchanges through history. This is particularly true for cultivars regarded as typical of southern Europe (Bacilieri et al. 2013; Laucou et al. 2018).

For medieval and modern times, direct relationships between modern and past varieties is clearly demonstrated by palaeogenomics, with the archaeological grape pip being most closely related to three native Georgian cultivars. Since grapevines are usually propagated by vegetative means from cuttings, it is possible for varieties to remain genetically unchanged for centuries, and this could therefore have led to exact matches with archaeological pips, as observed for a 'Savagnin Blanc' grape pip from medieval Orléans, France (Ramos-Madrigal et al. 2019). One might therefore anticipate that many relatively recent archaeological specimens, such as these historic Georgian samples, would produce exact genetic matches to modern grape varieties. While we found that one of the archaeological pips had a close similarity to a modern variety, our data were insufficient to determine if they were identical. It is intriguing that we did not observe more direct matches or close relationships between the other two pips and modern varieties. A possible explanation is that the GrapeReSeq database currently includes only 20 Georgian accessions, which is a small proportion of the country's 500+ named varieties (Maghradze et al. 2012) and these relationships might only be discovered through genotyping more accessions. Another possible explanation is that Georgian varieties have remained in flux through the centuries, as recent studies demonstrate extensive gene flow between wild and domesticated populations (Riaz et al. 2018).

GMM data reveal limited changes in the diversity of grape varieties cultivated over time, especially in comparison to the high morphological diversity recorded at each site. Identifying these possible changes would probably require more and larger samples.

Conclusions

The combined phenotypic and genetic study of some archaeobotanical grape remains from Georgia provide evidence that grapevines were used and cultivated in the country at least since the Late Bronze Age. This date seems recent compared with the much earlier (ca. 5800 BC) chemical evidence of wine making locally available and the regional archaeobotanical data showing grapevine cultivation since ca. 4000 BC. This apparent contrast is probably due to the fact that recent archaeological excavations and archaeobotanical studies in Georgia are still limited in number compared to other areas south of the Caucasus. Forthcoming investigations will probably change the situation considerably. Our study provides further evidence for the need to support research based on old samples with systematic radiocarbon dating, especially when uncharred plant remains are involved.

Our study combining GMM and aDNA provides the first insights into the history of grapevine diversity in a country with a very long wine-growing tradition that probably played a key role in the domestication of the species. Forthcoming archaeological excavations in the country should provide new waterlogged pip samples allowing the extension of palaeogenomic research to earlier periods.

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Pip shape echoes grapevine domestication history

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The pip, as the most common grapevine archaeological remain, is extensively used to document past viticulture dynamics. This paper uses state of the art morphological analyses to analyse the largest reference collection of modern pips to date, representative of the present-day diversity of the domesticated grapevine from Western Eurasia. We tested for a costructure between the form of the modern pips and the: destination use (table/wine), geographical origins, and populational labels obtained through two molecular approaches. Significant structuring is demonstrated for each of these cofactors and for the first time it is possible to infer properties of varieties without going through the parallel with modern varieties. These results provide a unique tool that can be applied to archaeological pips in order to reconstruct the spatio-temporal dynamics of grape diversity on a large scale and to better understand viticulture history. The models obtained were then used to infer the affiliations with archaeobotanical remains recovered in Mas de Vignoles XIV (Nîmes, France). The results show a twofold shift between the Late Iron Age and the Middle Ages, from table to wine grape varieties and from eastern to western origins which correlates with previous palaeogenomic results.

Today, grapevine (*Vitis vinifera* L. subsp. *vinifera*) is economically one of the most important cultivated fruit species in the world[1]. Its central economic and cultural role in the Mediterranean Basin goes back beyond the Greco-Roman era[2]. Modern genetics and archaeobotany concur in locating the origin of domesticated grapevine in the Near East, south of the Caucasus[3,4]. Its initial domestication is thought to have occurred during the Neolithic (between 6000 and 3000 BC) but the date is still debated. Chemical analyses of pottery vessels suggest that wine was already produced in the Caucasus area 8000–6000 years ago[2,5]. From its Near-Eastern cradle, viticulture spread to most of the Mediterranean and eventually the rest of modern-day Europe, between 3000 BC and 500 CE[2]. Viticulture could have started in Sardinia and Southern Italy as early as the late 2nd millennium BC[6] and in Southern Spain by the beginning of the 1st millennium BC, in connection with the Phoenician influence[7].

Grapevine has been dramatically modified and diversified since its early domestication. The most notable changes concern: the shift from dioecy in wild grapevines (*Vitis vinifera* subsp. *sylvestris*) to a hermaphroditic reproductive system for most of the varieties, the increase in berry and bunch sizes, the increase in sugar and acid content, and the variation in berry colour and shape[8,9]. These changes are so significant that the phenotypic diversity of the domestic grapevine, including its morphological component, is much greater than that of its wild counterpart[8]. Several thousand varieties can be distinguished[10], and are generally classified in two main groups: table (fruits consumed fresh or dried) and wine grapes.

Cultivated grapevine diversity is patrimonial and a direct product of its intertwined history with human societies. Because of this, cultivar diversity can help understand this shared history through the use of genetic or morphological markers.

This paper explores the global grapevine diversity through the analysis of seed morphology. It is known that seeds from wild and domesticated grapes differ in their form (i.e. size and shape); wild grapes produce roundish pips with short stalks and cultivated varieties produce more elongated pips with longer stalks[8]. Grape pips have long been a focal point in archaeobotanical studies, because of these well-known differences and because they often are the only remains that are preserved in archaeological contexts. Morphometrics, or the statistical description of shape, has a prominent place in the quantitative analysis of pips. Morphological characterization works on a highly integrated and well preserved datum, the pip shape, and its capacity to signal phenotypic resemblances, give major insights into domestication studies using modern and ancient material[11–14].

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Various pip measurements have been used to distinguish wild and domesticated *V. vinifera* subspecies in the archaeological record[15,16]. Recent quantifications of pip shape that use outline analyses have helped to improve the discrimination towards the identification of individual varieties[17–22].

The study of the whole phenotypic diversity in cultivated varieties reveals that two clear types can be discerned: table and wine varieties[23]. Table grapes tend to have large berries, sometimes seedless, and have relatively thin skin while wine grapes are smaller, have higher concentrations of sugar, are generally seeded, and have relatively thick skins[24].

Negrul[25–27] proposed a comprehensive classification of all the known varieties into three major groups or *proles*. *Proles orientalis* is composed of table grape varieties, with big berries, typical of the Near and Middle East and regions of the Mediterranean basin. *Proles occidentalis* gathers wine grape varieties from Central and Western Europe. They are typically more resistant to low temperatures and have smaller, more acidic berries with lower sugar content. The varieties of *Proles pontica* (Balkans, Black Sea and Caucasus) have intermediate characteristics between *orientalis* and *occidentalis*. They are mostly used for winemaking or for both table/wine purposes.

In recent years, the global diversity of cultivated grapevines has mostly been studied with the use of nuclear microsatellite markers[28–30], thousands of SNP markers[3,30,31], or both[32]. These studies tend to identify a global structure confirming the three major groups described by Negrul[25], as well as an additional group of Iberian varieties, and a number of varieties with admixed/intermediate assignment.

Using 20 microsatellite markers (SSR), Bacilieri et al.[30] were able to identify Negrul's three major groups, and a second level with two additional groups: "Iberian Peninsula and Maghreb" and "Table grapes from Italy and central Europe". In Lacombe's study[29], four distinct groups could be recognized using the same markers on a reduced set of varieties, in which closely related genotypes were excluded. In the fourth group, the Iberian cultivars were found to be associated to wine and table varieties from Asia Minor and the Caucasus. Laucou et al.'s findings[31], which were based on an array of 18k single nucleotide polymorphisms (SNP), present a similar organization into four main groups, with the Iberian and Negrul's groups, however, the majority of varieties were admixed.

This global structuring of *V. vinifera*, determined by its predominant use by humans and geographic origin, is a result of the long history of grape domestication and of the spread of viticulture. Negrul's hypothesis proposes that the main groups of cultivars were domesticated from different populations of wild grapevines[25]. The existence of secondary domestication events with local wild populations as opposed to mere introgression processes in other areas of the Mediterranean is still discussed, and may have helped shaped regional diversity[3,33,34]. The modern diversity of cultivated grapes stems from thousands of years of selection and diffusion through cuttings and seeds combining spontaneous hybridization and somatic variation[31].

Following morphometric analyses of grape leaves which demonstrate a weak correlation between leaf morphology and the East/West origin of grape varieties[35], we decided to use seed outline analysis to explore the structure of the diversity of cultivated varieties across the entire Eurasian and Mediterranean area. Our research aims to establish solid foundations on modern material, to further fuel archaeobotanical studies that can provide insights into past grapevine diversity and viticulture history. We used a representative collection of modern grapevine diversity in the form of a photographic pip shape collection, and we tested the reliability of discriminant models based on shape to infer: (i) destination use, (ii) geographical origins, (iii) conformity in the genetic structure found among varieties. Finally, we applied these models on archaeological remains as a first step into drawing finer-grained, morphological-based inferences about viticulture in the past.

Materials and methods

Reference collection of modern pips. This study includes 434 grapevine modern cultivars (Table A ESM). Their origins cover the entirety of Euro-Mediterranean diversity. Most of the cultivars were selected and sampled from the INRAE Grape Germplasm Repository (Marseillan-Plage, France). Additionally, autochthonous cultivars from the Caucasus area were sampled from the Saguramo Grape Repository (Jighaura, Georgia).

For each cultivar, 30 normally developed berries were randomly collected from a single, fully-ripe bunch. The final dataset comprised 12,346 pips.

Cofactors further used are presented in the Table A (ESM) and summarised in the Table B (ESM). They comprised: berry size, geographic origins, and destination use assessed from general bibliography[10,36]. We also included genetic assignation[30,31]. These two studies and the present one largely used the same set of varieties. However, because these sets were compiled at different time and with different aims, information may be missing, debated or unknown, for any given variety (Table A). For our analyses, only cultivars with well-defined information were used (Tables A, B). Berry size was observed and recorded over several years in the grapevine repositories and coded according to the International Organization of Vine and Wine descriptors[37].

Archaeological material. As a case study, we selected the site of Mas de Vignoles XIV (Nîmes, France) where large quantities of well-preserved, waterlogged, grape pips dating back to two time-periods (Late Iron Age/Early Roman and Medieval times) were found in association with other plant remains.

The site is located in the alluvial plain of the river Vistre and was excavated by INRAP[38]. The site was occupied at different periods between the Neolithic and the early Middle Ages. The first traces of occupation are very sporadic, but from the end of the Bronze Age onwards, the area appears to undergo continuous changes in land use, mainly oriented towards agricultural and craft activities and including few traces of human habitation. During the second century BCE (late Iron Age or Republican period) a large farmhouse was identified nearby; the remains of its northern boundary were uncovered at Mas de Vignoles XIV. This farmhouse was later replaced by two small farms surrounded by areas devoted to agriculture and animal husbandry. The pips from this period were recovered from a well (PT14203, SU14258) and a ditch (FO14194, SU14152) that was part of a network of ditches delimiting the farming areas.

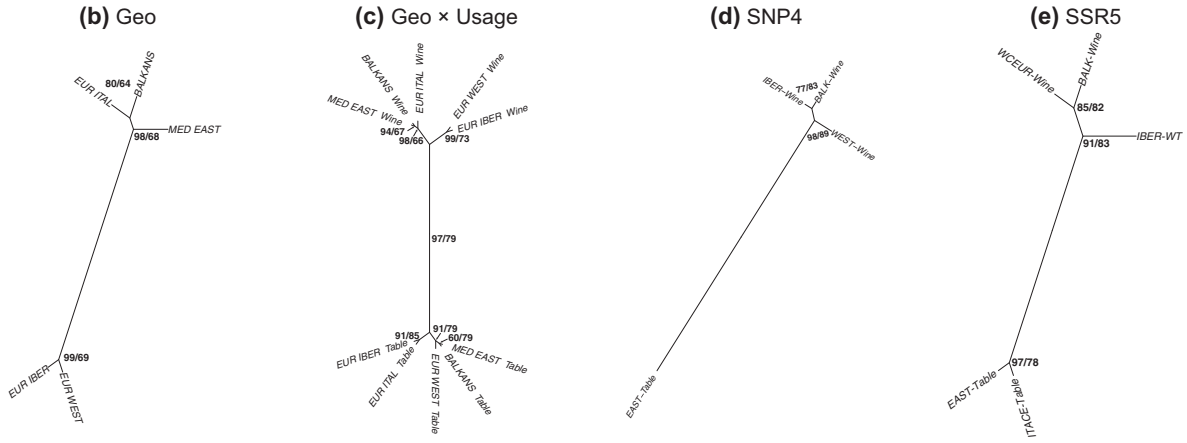

Figure 1. Unrooted trees obtained with hierarchical clustering of the form of pips (length + shape) grouped according to covariates of interest. For each node the numbers correspond to pvclust/cross-validation values. Tree (**a**) corresponding to use, as a two-class case has no topology and is not presented.

Archaeological remains from the Middle Ages are not abundant, nor well preserved and include very few traces of human habitation; this suggests that people lived further away, probably due to the unfavourable topography and edaphic conditions (depression area with high water table). The archaeological structures found include several ditches, wells, storage pits, animal enclosures and wooden constructions dedicated to farming activities ; the importance of animal husbandry is suggested by the abundance of cattle remains, which is unusual in a region mainly dominated by sheep/goat, but in agreement with local conditions[39]. The presence of beetles associated with stable areas further reinforce the evidence of animal husbandry. Flax and hemp figure among the potentially cultivated plants, other than grapevine. The *Vitis* pips investigated come from a well (PT12024, SU12109 and 12111) radiocarbon-dated to the Early Middle Ages: Poz-48697: 1200 ± 30 BP (706–945 cal AD[40]).

Nine pips from Mas de Vignoles XIV previously delivered aDNA results showing that varieties of different origins may have been cultivated during the Late Iron Age and the Middle Ages[41].

Pip morphometric description. Each pip was photographed according to two orthogonal views (dorsal and lateral) by the same operator (TP). Outline coordinates (x; y) were extracted from these images and two markers (one at each tip of the pips) were used to normalize the position, size, rotation and first point of the outlines by registering them on "Bookstein coordinates", that are (x = − 0.5; y = 0) and (x = 0.5; y = 0) coordinate points. For each view, elliptical Fourier transforms were used to convert the contour geometry into "Fourier coefficients". Elliptical Fourier transforms are detailed elsewhere[42,43]. The number of harmonics was chosen to gather 95% of the total harmonic power[43], which corresponds to five for both views. In terms of operator error (e.g. while positioning the pip), this is less harmonics, and thus a conservative choice, compared to previous recommendations of six harmonics for both views[22]. With four coefficients per harmonic, 40 coefficients were obtained and further used as quantitative variables describing the shape. Pip length was derived from outline coordinates. Pip length was shown to be the best predictor of all other lengths measured on pips[20] and here helped to analyse form, that is the shape plus size. When compared to manual measurements obtained in a subset of another study[20], error was centred and was, on average, below 1% (~ 1/20 mm). The correlation between the berry and the pip size previously shown[20] was here tested on a larger dataset using one-tail Wilcoxon rank tests (medium vs. small, large vs. small; Fig. A ESM). The final matrix analysed and used in models was thus [12346 × 41].

Statistical environment. Analyses were performed in R 4.0.2[44], with the packages Momocs 1.3.2[43] for everything morphometrics, MASS 7.3-51.6[45] for linear discriminant analyses, tidyverse 1.2.1[46] for general data manipulation and visualization, pvclust 2.2-0[47] for assessing uncertainties in hierarchical clustering and ape 5.0[48] for unrooted tree representation.

Visualizing and testing for use, geographical and genetic signals. First, a principal component analysis was calculated on the full matrix of coefficients to visualize how each level of each cofactor of interest were located in this synthetic morphological space (Fig. B ESM).

To test for a costructure between pip form and cofactors, two approaches were used: hierarchical clustering with robustness assessment, and cross-validation using permutational and balanced linear discriminant analyses. To assess geographical structuration the (putative) countries of origin of cultivars were organized in geographical groups (Table A ESM, Table B ESM).

For hierarchical clustering, the averaged coefficients for each level were used to calculate a distance matrix using correlation as the distance method, on which a hierarchical clustering using average (i.e. UPGMA) was calculated. Topologies obtained were presented as unrooted trees (Figs. 1, C ESM). The robustness of nodes was

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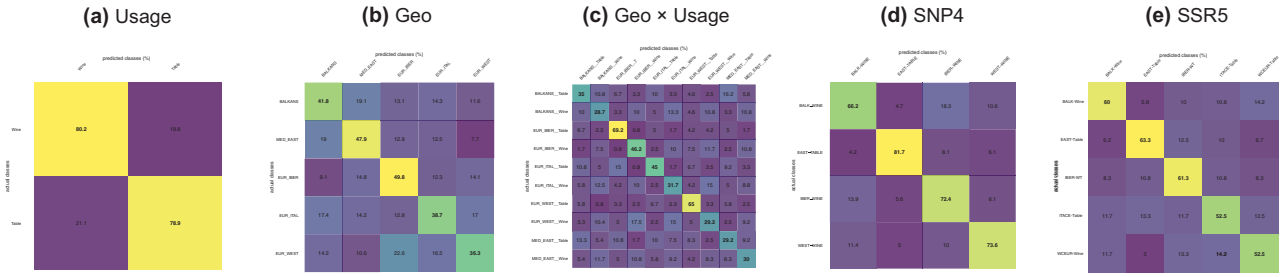

Figure 2. Confusion matrices for discriminant analyses. Cells present median percentages obtained over 100 permutations of balanced datasets. Along the diagonal, values in bold indicate significant values (i.e. above the maximal value obtained by chance alone among 100 permutations).

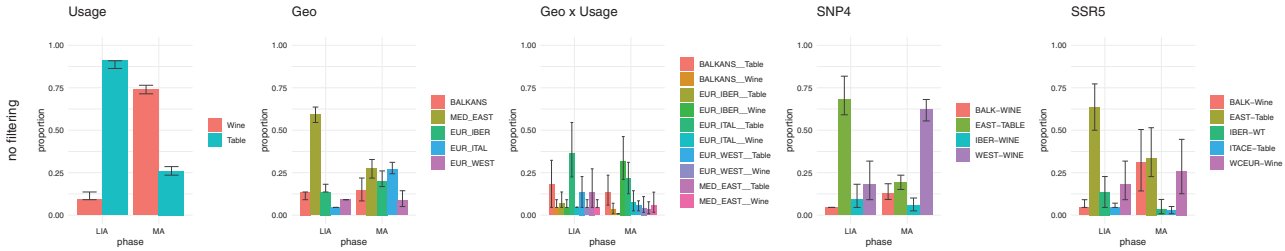

Figure 3. Inferences for archaeological pips of the domesticated type from the Mas de Vignoles XIV. The columns correspond to the different models presented after an inference on the wild/domesticated. The proportion are presented without filtering (see also Fig. F ESM).

estimated through multiscale resampling[47] with proportions ranging from r = 0.5 to r = 1.4 with a 0.1 increment and 10^2 resampling with replacement. The "approximately unbiased p-values" were retained[49] and presented in Figs. 1 and C (ESM).

This method had the merit of simplicity but did not accounted for: (a) variability within groups, (b) overlapping between groups, and (c) unbalanced groups sizes since coefficients were averaged. Moreover, for archaeobotanical inference, we need predictive models along with their performance assessment. We thus combined this approach with linear discriminant analyses (LDA). Class accuracies (i.e. tree leaves) were presented as confusion matrices, and class clustering (i.e. tree nodes) were estimated using classes belonging to a node against all others. The index used is accuracy (the proportion of correctly classified pips), using leave-one-out cross-validation. To cope with unbalanced sample sizes, 10^2 permutations were used[50], and each sampled the minimal group size among all groups. The median value was reported on each node in Fig. 1. For each model, we also presented the confusion matrices obtained for each group against others (Fig. 2), as well as the distribution of accuracies obtained under this null model (here obtained through simulations but expected to follow a multinomial distribution; Fig. D ESM).

Assessing filtering predictions. In a "predictive" LDA, new statistical individuals are always assigned to the class with the highest posterior probability. In filtering out predictions based on posterior probabilities sample size is exchanged for identification confidence. The posterior probability cut-off value is often based on rule of thumb by picking an arbitrary threshold. Here we use a twofold approach, combining LDA, resampling and filtering in the spirit of[50]. Across the 10^2 permutations, we calculated the class accuracies and the proportion of the original group sample size retained, as functions of cut-off value for posterior probability (Fig. E ESM and Table C ESM). We also explored the same relationship using the proportion of cases among permutations where each pip was attributed to a given class as a cut-off value (Fig. E ESM and Table D).

Inferences on archaeological material. Former studies have shown that archaeobotanical assemblages generally include an important proportion of wild type grape pips[16,22]. For this reason, we used a first LDA_{status} to identify domesticated and wild type pips with the dataset published[17,51]. Here, we used the same approach with 10^2 balanced permutations. For each pip, the majority rule applied. In the cited studies, these LDA achieved 95% accuracy (without filtering) in distinguishing between pips from wild grapevine individuals and those from domesticated varieties. Further inferences about archaeological pips identified as the domesticated type were then obtained using the 10^2 balanced models previously presented (Figs. 3 and F ESM): we inferred destination use (LDA_{Use}), geographical origins (LDA_{Geo}), the destination and geographical origins jointly ($LDA_{Geo \times Use}$) and genetical grouping (LDA_{SNP4}, LDA_{SSR5}). Inferred proportions of each class are presented with three approaches: no filtering (Fig. 3), filtering out pips with a median posterior probability observed among permutations < 0.8 (Fig. F ESM); filtering out pips that were attributed to the same class less than 50% of the time (Fig. F ESM).

Results

Berry size. Our results show that berry size in modern grapes positively correlates with pip size (Fig. A ESM): varieties with medium-sized berries had longer pips than those with small-sized berries (Wilcoxon rank tests: $W = 6{,}581{,}329$, $P < 10^{-16}$). Similarly, large-sized berries had longer pips than those with medium-sized berries ($W = 11{,}851{,}551$, $P < 10^{-16}$).

Principal component analysis. The first two components of the PCA obtained from the full matrix of coefficients captured 73.2% of the total variance in form. For the sake of clarity, for each variety only the PC1-PC2 centroid was displayed (Fig. B ESM). With the exception of "Use", which shows a clear positional difference between table and wine varieties, other cofactors of interests showed more subtle contrasts.

Form and destination use. After discarding mixed destination varieties, the remaining set included 3,106 pips (Table B ESM). The discriminant model achieved a good discrimination rate (wine = 80.2%; table = 78.9%—Fig. 2), far better than the results expected of chance alone (expected = 50%; max. observed for 100 permutations = 53%—Figs. 2 and D ESM).

Form and geographical origin. The first geographical model, based on the putative origin of cultivars according to our bibliography, included all regions except NEW WORLD. For each group, 480 pips were included. The resulting tree (Fig. C ESM) showed a clear geographical structuring with two separate clusters (EUR_IBER + EUR_WEST; EUR_ITAL + BALKANS + MED_SOUTH + MED_EAST) with EUR_EAST in between. Varieties gathered in the ASIA_CENT group were clearly set apart. For the other nodes, pvclust values were all > 94 and cross-validation > 64%. Class accuracies ranged between 23% (MED_EAST) and 63% (ASIA_CENT); aside from MED_EAST, all were better than chance alone (expected = 12.5%; $max_{100} = 25\%$).

A second geographical model was restricted to "core" historical regions (Fig. 1). In practical terms, the varietal sampling within these groups was more exhaustive and allowed to include 1,439 pips in permutations (Table B ESM). The resulting tree (Fig. 1) clearly distinguished between EUR_IBER + EUR_WEST and the other groups. All nodes presented pvclust values > 80 and cv values > 64%. Class accuracies ranged between 36% (EUR_WEST) and 50% (EUR_IBER), all better than chance alone (expected 20%; $max_{100} = 29\%$—Figs. 2 and D ESM).

With fewer groups, one usually expects better accuracies; since this was not the case, we suspected a latent effect. We thus built a third model including the same core geographical regions, and combined them with their destination use (Fig. 1). Due to the lower number of table varieties, only 120 pips were included in each permutation (Table B ESM). Despite this, a clear structure with two neat clades corresponding to destination use were observed. Apart from the clade representing BALKANS_Table and MED_EAST_Table, all nodes had pvclust values > 91 and cv values > 66% (Fig. 2). For the wine varieties, BALKANS and MED_EAST were clustered together (pvclust = 94; cv = 67%), then with ITAL (pvclust = 98; cv = 66%). Among wine varieties, another clade grouped EUR_WEST and EUR_IBER (pvclust = 99; cv = 73%). Class accuracies ranged between 29% (BALKANS_Wine, EUR_WEST_Wine, MED_EAST_Table) and 69% (EUR_IBER_Table), overall, far better than chance alone (expected = 10%; $max_{100} = 22\%$—Figs. 2 and D ESM).

Form and genetic structure. We then explored whether the genetic structure found using SSR[30] and SNP[31] data was also echoed in pip form. The first model used SNP4 (Fig. 1) and included 360 pips in each permutation. The EAST_TABLE group was distinguishable from the three other groups (pvclust = 98; cv = 89%), and in the latter BALK_Wine and IBER_Wine clustered together (pvclust = 77; cv = 83%). Cross-validation values for each node were all > 83%. Class accuracies for each group ranged from 66% (BALK_Wine) to 82% (EAST_Table), much better than chance alone (expected = 25%; $max_{100} = 38\%$—Figs. 2 and D ESM).

The second model used microsatellite data (SSR5) and led to similar results. This model used only 120 pips for each permutation and distinguished two groups EAST_Table and ITACE_Table, and WCEUR_Wine + BALK_Wine + IBER_WT. All nodes presented pvclust values > 85 and cv values > 78% (Fig. 1). Class accuracies ranged from 53% (ITACE-Table and WCEUR-Wine) to 63% (EAST-Table), again, better than chance alone (expected = 20%; $max_{100} = 35\%$—Figs. 2 and D ESM). We built a final model excluding ITACE_Table (not shown), which allowed us to increase the number of pips to 420. The same topology was obtained among remaining groups, all pvclust values were > 77 and cv values > 70%, and nodes and class accuracies for groups ranged from 67 to 71%, much better than chance alone (expected = 25%; $max_{100} = 36\%$).

Sample size and filtering out based on posterior probabilities. As expected, filtering results based on posterior probabilities improves class accuracies at the cost of reduced sample sizes (Fig. E ESM, Tables C ESM and D ESM). The models with the lowest class accuracies before any filtering were the ones with the steepest slopes for the proportion of filtered out curves. Such simulations are useful since they show that with low class accuracies and without filtering on posterior probabilities, the benefit in the accuracy gain is quite low compared to the price to pay in terms of sample size reduction. For instance, when filtering at a posterior probability of 0.5, the absolute gain is only 11% (33% of relative gain) but 64% of the original sample size is filtered out. Also, the models with the smallest number of pips in each permutation showed the highest uncertainties for class accuracy estimates. Due to the resampling nature of these simulations, the higher the number of pips, the lower the variation expected for the estimates.

Application to the archaeological material. LDA_{status} trained on the reference material led to 95.2% accuracy for both wild and domesticated types (100 permutation using 2005 pips). When applied to archaeologi-

cal material, LDA_{status} classified 81% pips (102/128) and 39% (74/204) pips as the wild morphological type for the Late Iron age (LIA) and the Middle Age (MA) phases. These pips were discarded from further analyses and the remaining sample sizes were thus 26 and 130 for LIA and MA phases. Overall, the different filtering approaches led to congruent results (Figs. 3, F ESM). We tried a high pass of 0.8 for posterior probabilities whose results on archaeological material (not shown) confirmed those obtained on modern material: sample sizes were dramatically reduced and results were much more dependent on the training set. The overall tendencies observed for the Mas de Vignoles XIV and based on pip shape were a shift from table to wine type, and from Southwest Asia to Western Europe, between the LIA and MA. The cofactors including use in their definitions (Use, Geo × Use, SNP4 and SSR5) all corroborated the predominance of the table type during the LIA and of the wine type during the MA. Similarly, for the geographical origins, all models provide evidence of the Eastern origins for the LIA and of Western European origins for the MA (Figs. 3, F ESM). The length of the pips classified in the domesticated-type of the LIA assemblage is greater than that of the MA. Consequently, the berry size that can be inferred is higher for the LIA, close to medium-size to large modern berries, while the size inferred for MA berries is very small (Fig. A ESM).

Discussion

The destination use (table/wine) and geographical origins of *V. vinifera* are echoed in the shape of modern grapevine pips and corroborate the structure found using genetic markers. The results here obtained from this modern material dataset pave the way for a more comprehensive archaeobotanical analysis of the grapevine historical agrobiodiversity and biogeography.

Analyses of genomic sequences brought direct insights into *V. vinifera* genealogies, kinships and, more generally, into the intraspecific structuring of the domesticated grapevine[3,4,30,31,52,53]. Genetic markers are direct, sensitive and accurate proxies, but do not provide clear-cut groups within agrobiodiversity since variety amelioration is the product of a continuous and intertwined history and are rarely performed on the large spatio-temporal scale offered by morphometric studies. The two studies where genetic assignation were used demonstrate a high proportion of admixed varieties[30,31].

Pip shape, on the other hand, integrates genotypic, developmental and environmental factors. As a phenotypical trait, it is known to be a much more indirect proxy for measuring and uncovering agrobiodiversity structuring. Because morphology is prone to homoplasy, two identical shapes may not be directly genetically related and may instead reflect a potentially mixed signal of ancestry, similar environmental adaptations, as well as non-adaptive natural processes (i.e. drift). Our results are validated by molecular approaches rather than *confirming* them. Under certain conditions, for example where a strong population structure and divergent selection are present, phenotypical approaches may be superior to molecular ones for measuring agrobiodiversity[54].

Our results show that grapevine pip diversity is significantly structured by use and, to a lesser extent, by the geographical origins of varieties. Use had the best class accuracies; the pips of wine and table grapevine varieties have different form. This was shown in a previous study, which used a smaller set of varieties[20]. Several other studies based on phenotypic[23,25] and genetic markers[3,30,31], have already concluded that grape varieties were structured, above all, according to their use as table or wine.

More importantly, our results also established a significant geographical correlation in the shape of pips. Between the two genetic models, the classifications from SNP4 gave better class accuracies for pips than those of SSR5. The fact that SNP4 was calculated using 10,000 SNP markers scattered along each chromosome, bolsters the findings of our study since the SSR5, only used 20 microsatellite markers. Despite having one class less, the SNP4 dataset may be more representative of pip variability since it was trained using more varieties, and thus more pips in permutations.

The large-scale structure in pip shape reflects the same blurred boundaries as those reported by genomic analyses. In genetic analyses, unassigned varieties are mostly attributed to human-assisted movement of cultivars across regions and inter-group breeding. In morphologic analysis, however, additional factors may be involved. i.e. environment and development constraints as well as homoplasy. Nevertheless, the morphology-based geographical tree indicates the clustering of eastern groups and western groups, and Italian varieties clustering with eastern ones. The same pattern is found for the tree combining geography and use, where despite a predominant Use structure, the Italian wine varieties are clustered with eastern ones.

Interestingly, incorrect assignations may also reveal meaningful information. Misclassified seeds fall primarily into groups that are closely related in terms of use and geographical origin. For instance, in the Geographical confusion matrix, the eastern Mediterranean group is most frequently misclassified with pips of the "Balkans" groups, and vice-versa. On the same confusion matrix, the "EUR-ITAL" group reflects its intermediate nature between western and eastern varieties. For these reasons, retrieving congruent results using shape alone was far from a foregone conclusion.

Finally, it is worth noting that the *proles* classification proposed by Negrul[25] was based on morphological criteria and was later confirmed by genetical studies[30,31]. These different approaches are largely congruent and provide evidence that grapevine diversity is not only structured, but that its structuring is related to, and likely a product of, the history and the geography of viticulture.

Dedicated genotype-to-phenotype association studies could help decipher the mechanisms behind such correlation between the shape of pips and the cofactors of destination use and geographical origins. The destination use is directly related to the phenotypic traits and chiefly those of the berry (e.g. size, flavors, aromas, etc.). Berry trait loci have been reported in other studies[32,55], as have the covariation between the berry and the pip size and shape[20]. With regard to geographical origins, we cannot exclude an indirect link with climatic conditions through crossing of varieties from different origins but we see no reason why a particular geographical origin may directly select a particular pip shape. Berry size likely has a correlation with geographical origin because

of the relationship between use and geography. Negrul[25] already highlighted the ubiquity of large-berry table varieties in the Near-Middle East, that of small-berry wine varieties in Central-Western Europe, and intermediate varieties in his *proles pontica* (Balkans, Caucasus, Black Sea). Overall, it is likely that pip shape was not directly implicated in selection and that the subtle changes in its shape are probably neutral. One can thus reasonably hypothesize that any shape change is therefore caused by genetic drift and/or genetic linkage.

While indirect, both in origin and signature, pip shape is informative about variety origins and use. Shape is also often the only exploitable datum on archaeological remains, and these results are therefore of prime interest to help us better understand grapevine agrobiodiversity through both time and space[16,17,21].

Archaeobotanical inference is mostly actualistic: insights obtained from modern material generate inferences for archaeological remains. So far, the shape of pips has been used to distinguish between wild and domesticated types[15,16,22] and, more recently, to identify domesticated morphotypes that correlate with modern varieties[17,21]. The identification of similar modern varieties amongst a diverse subset can be used to make infraspecific conclusions if their properties are compared to those of the already identified varieties[21,51].

In our study, we used a more extensive and more representative collection of modern seeds, the largest worldwide, to the best of our knowledge. The pioneer collection used in Terral et al.[22] increased since then[17,51,56]. Here, we moreover directly infer cofactors of interest without the intermediate identification of the most-resembling modern variety. This is an important result because it means that properties of varieties cultivated in the distant past can be inferred directly from the archaeological remains without having to compare them with modern varieties that may not be appropriate counterparts.

To increase the robustness of inferences, predictions are sometimes filtered out based on posterior probabilities[12,51]. When cut-off thresholds are selected by the "rule of thumb" method, the trade-off between accuracy and sample size is often forgotten. Our results indicated that this approach should be used sparingly and only when classification accuracies were already proven to be better than classification by chance alone. One should also keep in mind that poorly classified pips may actually be "true" intermediate forms, and if filtered out, may accentuate the contrast between or within assemblages. Finally, when using linear discriminant analyses, particularly when groups have significantly unbalanced sample sizes, the use of permutations is preferable for obtaining better estimates of their accuracies[50].

We applied the models trained on modern material to the Mas de Vignoles XIV assemblages whose palaeogenomic information identifying the relationships between ancient grapes and modern diversity had already been obtained[41]. It is an ideal opportunity to combine aDNA approaches with and morphometrics strike force, although not on the same pips.

Our morphometric analyses of the Mas de Vignoles XIV assemblage demonstrate notable changes in viticulture between the Late Iron Age and Middle Age. The first one was the decreased prevalence of the wild type, from a predominant to a minority proportion. This decrease was previously observed in the study of several other archaeological sites in southern France[16]. It can be assumed that this wild type, which is phenotypically different from modern varieties, corresponds to a part of the diversity amongst historically cultivated varieties rather than being gathered wild berries[16].

Changes were also observed in domesticated pips. The different models agree on a twofold trend "between" the LIA and the MA: a shift from table to wine varieties, and from eastern to western varieties. These changes are probably two facets of the same shift. The pip lengths are also much smaller for the Middle Age assemblages. This direct measure is congruent with the LDA results as well as those of a previous study, which showed that wine varieties have shorter pips than table ones[20]. The SSR4 model, that should be considered more robust than the SSR5 as discussed above, similarly exhibits a contrast between these two historical phases: the predominance of Eastern table varieties in the LIA and of Western wine varieties in the MA. According to aDNA results, the three seeds studied from the LIA sample resemble table grapes as well as Eastern and Iberian wine varieties whereas medieval seeds are more similar to Western European wine varieties[41]. Although the seeds used were not the same as those of our study, they nevertheless corroborate our observations of a geographical shift towards Western wine varieties.

It is not possible to prove with certainty that the grape seeds found at Mas de Vignoles XIV came from locally grown grapevines, but its rural location may suggest this. While grapes were traded and could be transported over long distances in Roman times[57], archaeological findings have revealed traces of grapevine cultivation on the outskirts of the city of Nîmes, in the close vicinity of Mas de Vignoles XIV, from the second century BC onwards[58]. Until recently, it was believed that by the late Iron Age the vines cultivated in the South of France were only intended for wine production[59]. However, while vineyards appeared to be fairly extensive around the city of Nîmes during the early Roman period, wine cellars and wine production equipment were not very widespread in the excavated settlements[58]. This observation could be consistent with the hypothesis that parts of vineyards were dedicated to the cultivation of table grapes. These new results allow us to imagine the possibility of a viticulture partly destined for table purposes using eastern varieties rather than native or locally domesticated varieties.

Data availability

Full datasets will be released upon acceptance. They are (privately) available there: https://figshare.com/s/7578a0740dfbac9e3742.

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Author contributions

Conceptualization: L.B., J.F.T., V.B., T.L., R.B.; Data curation: S.I., T.L., T.P., V.B., C.P., R.B., J.F.T., L.B.; Formal analysis: V.B., A.E.; Funding acquisition: L.B., A.E.; Investigation: V.B., S.I., T.P., J.F.T., L.B.; Methodology: V.B.; Project administration: L.B., J.F.T.; Resources: T.L., C.M., D.M., I.F., H.P.; Software : V.B.; Visualization: V.B.; Writing—original draft: V.B., L.B.; Writing—review and editing: V.B., L.B., J.F.T., A.E., T.L., R.B., I.F.

Competing interests

The authors declare no competing interests.

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Chapter 3

Study in Georgia

Viticulture and winemaking in Georgia

CHKHARTISHVILI N., MAGHRADZE D.

In: Maghradze D., Rustioni L., Turok J., Scienza A., Failla O. (Eds). Caucasus and Northern Black Sea Region Ampelography. *Vitis* (Special issue). 2012. Pp. 169-176.

Wild grapevine

Wild Grapevine *Vitis vinifera* ssp. *sylvestris* Gmel., the supposed wild ancestor of the cultivated grapevine *Vitis vinifera* ssp. *sativa* D.C., is a typical representative of the Caucasian and Georgian flora. It grows sporadically in woods, forests, lowlands and rivers' banks up to 1,200 m a.s.l. (Ramishvili 1988). The history of the wild grapevine in Georgia should be separated in two periods: I) since the earliest time until the second part of 19th century; II) since the 1860s until today, when Oidium, Mildew and Phylloxera, together with industrial and urban expansion, destroyed spontaneous development of wild grapevine populations.

The first researcher who started investigations of the wild grapevine of Georgia was F. A.Kollenati (1846), followed by F. Ruprecht (1869), N. Sredinskii (1874), A. DeCandol (1883), I. Planschen (1887), V. Lipskii (1885), S. Timofeev (1892), G. Radde (1901), D. Sosnovskii (1925, 1946), N. Vavilov (1931), R. Ergesian (1946), R. Burkach-Abramovich (1953), M. Ramishvili (1943, 1948, 1968), L. Pruidze (1966), E. Chamagua (1968), R. Ramishvili (1988, 2001) and others (Ramishvili 1988).

Fig. 10: Wild grapevine *V. vinifera* ssp. *sylvestris* Gmel. is a typical representative of Georgian flora. It grows manly in river gorges. It has undergone scientific evaluation since 1846 (Kollenati 1846).

R. Ramishvili investigated wildly growing grapevines of Georgia in the second half of the 20th century (1956-1988). He collected about 400 genotypes in a field collection. On the basis of the results, he wrote the book "Wildly Growing Grapevine of the Trans-Caucasus" (1988). According to R. Ramishvili, there are three types of wildly growing grapevines in Georgia: 1. real *V. vinifera* ssp. *sylvestris* Gmel.; 2. feral varieties *V. vinifera* ssp. *sativa* D.C.; and 3. intermediate forms between these two types, named as V. *vinifera* ssp. *silvesatis* Ram. On these bases, we have a map of the spreading of wild grapevine in 8 main concentration centres.

The institute of Horticulture, Viticulture and Oenology (IHVO) could renovate investigation, description and inventory of wild grapevines in the framework of the above mentioned projects (Chkhartishvili *et al.* 2005, Maghradze *et al.* 2006). Fifty populations of wild grapevine with 180 plants were described; size of population varied from 1 to 20 plants, with an average quantity of 3.8 plants per site. According to the number of plants of the populations, they were classified as "Very bad" (64 %) or 'Bad' (24 %) and only 12 % - as "Regular".

Thanks to the Geographic Information System (GPS), it has been possible to describe the plants in their location. Harmonized ampelographic descriptors of OIV (1983, 2007), IPGRI (1997) and GENRES 081 descriptors were used for ampelographic, agronomic and cytological characterization of vine organs. Antocyanin analysis was made by HPLC technique. In 2008, a joint research with Spain (University of Sevilla) was organised for investigating the sanitary status of 10 wild grapevine *in-situ* populations, describing plant associations to wild vines. Twenty-two genotypes of wild vine together with 139 autochthonous varieties were characterized by 20 SSR markers, demonstrating that wild accessions are well distinguished from the cultivated compartments. Forty-six selected exemplars of wild grapevine and 7 genotypes of vine collected by R. Ramishvili were propagated at the University of Milan for collection, while just several forms are in *ex-situ* preservation in Telavi grapevine collection and Tbilisi Botanical Garden.

Despite being so widespread in the past, *V. vinifera* ssp. *sylvestris* is now included in the "Red Book of Georgia"(1982) for *in-situ* preservation. Only few populations are available in the reserve areas of Georgia and the activities for preservation of *V. vinifera* ssp. *sylvestris* in other areas are not sufficient.

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Wild grapevine in Georgia

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Abstract

Wild grapevine *Vitis vinifera* ssp. *sylvestris* Gmel. from Georgia is particularly interesting due to well-known contribution of the Caucasus region in domestication of cultivated grapevine in the Old World. That is why ample program of investigation for wild grapevine of Georgia have been initiated since 2004 within frameworks of international projects or institutional collaborations. Some results of these research activities are reported in this article, demonstrated progress in inventory and evaluation of wild populations; ampelographic, cytological and chemical characterisation of discovered plants; propagation of wild vines for its father conservation.

Introduction

Wild Grapevine *Vitis vinifera* ssp. *sylvestris* Gmel., the supposed wild ancestor of the cultivated grapevine *Vitis vinifera* ssp. *sativa* D.C., is a typical representative of the flora of the Caucasus and Georgia among it. This plant is a lodger of almost all woody regions, most part in forests on lowlands and rivers' banks up to 1200 m above sea level in Georgia (Ramishvili, 1988). It grows sporadically on the territory of the country.

Investigation of wild grapevine of Georgia together with local germplasm of autochthonous varieties has particular scientific interest as the South Caucasus area is considered, according to the primary De Candolle's and Vavilov's hypotheses as one of the main centers of origin and domestication for cultivated grapevine *V. vinifera* L. *sativa* DC. (Vavilov, 1926, 1931; McGovern, 2003; Forni, 2005, 2006; Costantini *et al.*, 2005-2006). Archaeological excavations, dating back to the VI-IV Millennium BC, have evidenced the existence of grape seeds and other plant remains, widely dispersed in Georgia, combined with materials relating to viticulture and winemaking activities (Kighuradze, 2000; Ramishvili, 2001; Rusishvili, 2010). These evidences are well supported by the existence of wild populations of *V. vinifera* L. *sylvestris* Gmel. in Georgia (Ramishvili, 1988) and by the huge number of

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autochthonous varieties, represented by a total of 525 varieties with black, white, red, rose and grey grapes used for wine-making or table consumption (Ketskhoveli *et al*., 1960). Thus, it is believed that wild grapevine has provided an important initial impulse to the domestication of grapevine, by the old civilisations here.

Beside the conservation, the Georgian grapevine germplasm is the object of intensive investigations in various scientific fields; it attracts international collaborations because of its genetic diversity (Vouillamoz *et. al*., 2006; Maghradze *et al*., 2009; Maghradze *et al*., 2010a, Schaal *et al*, 2010). Investigations based on SSR fingerprinting and modern ampelographic methods are used for understanding their genetic structure and the phylogenic relationships with the World's germplasm.

Grapevine as a plant has long time history in Georgia. According to Ramishvili (2001), in the "Catalogue of Excavated Plants" (1973), including some representatives of Neogenic and the Fourth Period flora from Georgia, are also includes fossils of *V. silvestris* Gmel.

History of wild grapevine in Georgia should be separated in two periods: I) since the earliest period until the second part of XIX centuries, when there was the best conditions for growing of this plant here; and II) since 60th of XIX century until today, when fungal diseases (*Oidium* and *Mildium*) and *Phylloxera,* and plus expanded human activities, destroyed spontaneous development of wild grapevine populations.

In limited number medieval references present some information about existing of wild grapevine on the territory of Georgia (Sharden, 1711). But the first researcher, who started investigation and made systematization of the wild grapevine of Georgia, was Fr. A. Kollenati (1846). Following him, wild grapevine of Georgia and the Caucasus have been investigated by: F. Ruprecht (1869), A. DeCandol (1883), V. Lipskii (1885), G. Radde (1901), D. Sosnovskii (1925, 1946), N. Vavilov (1931), R. Ergesian (1946), R. Burkach-Abramovich (1953), M. Ramishvili (1943, 1948, 1961), L. Pruidze (1966), E. Chamagua (1968), R. Ramishvili (1978, 1988a, 1988b, 2001), N. Cholokashvili (1983), M. Amanov (2006), and others.

R. Ramishvili investigated wildly growing grapevine of Georgia in the second half of XX century (1956-1988). He has organized research

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expeditions almost in all regions of the country and collected about 400 genotypes in a field collection. On the obtained results he has written the book "Wildly Growing Grapevine of the Trans-Caucasus" (1988). According to R. Ramishvili three types of wildly growing grapevine present in Georgia: 1. Real *V. vinifera* ssp. *sylvestris* Gmel.; 2. Running wild cultivated varieties *V. vinifera* ssp. *sativa* D.C.; and 3. Intermediate forms between these two types, named as V. *vinifera* ssp. *silvesatis* Ram.

Based on investigation of the XX century a map for spreading of wild grapevine in Georgia in its 8 main centres of concentration has been singled out.

The Institute of Horticulture, Vitisculture and Oenology (Tbilisi, Georgia) could renovate investigation and re-inventory of wildly growing grapevine of Georgia in the framework of the international project of Biodiversity International "Conservation and use grapevine genetic resources in the Caucasus and Northern Black Sea region" in the period of 2003-2008. Activities in this field covered inventory, description, and investigation of wild vines (Chkhartishvili *et al.,* 2005; Maghradze *et al*., 2006). Investigation was continued in the frameworks of the other international projects also like French "ECO-NET" project in the period of 2006-2007 and the EU "Grapegen06" project in 2007-2010.

The goal of this work was multidisciplinary characterization and evaluation of Georgian wild vine gene pool by using modern techniques of ampelography and molecular genetics with association of native varieties of Georgia.

The particular targets were following: gathering available information on wild vine in Georgia; inventory of wild population in nature and evaluation of their status; ampelographic and ampelometric investigation of discovered wild plants based on OIV and IPGRI descriptors; DNA fingerprinting to detect genetic variation with usage of 20 Simple Sequence Repeats (Microsatellites) (SSR) markers; verification of wild samples and germplasm of native varieties; evaluation of sanitary status of wild populations; activities for *ex-situ* preservation of newly discovered and recovered samples of wild grapevine.

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Evidence of the ancient viticulture

On the map of McGovern (2003) (Fig. 1) is indicated Georgia as a place of wild grapevine location and a place of important archaeological findings linked with viticulture.

The VII millennium B.C. is considered to have the initial stage of setting habitation in Georgia. Palaeobotanical and archaeological data of this period are well evidence on the site of the so-called '*Shulaveri-Shomu tepe*' culture, dated to the VI and V millennium BC. By archaeologist excavation were founded very many botanical remains and other types of material remains (the sow of a deer horn, grains of wheat and barley, seeds

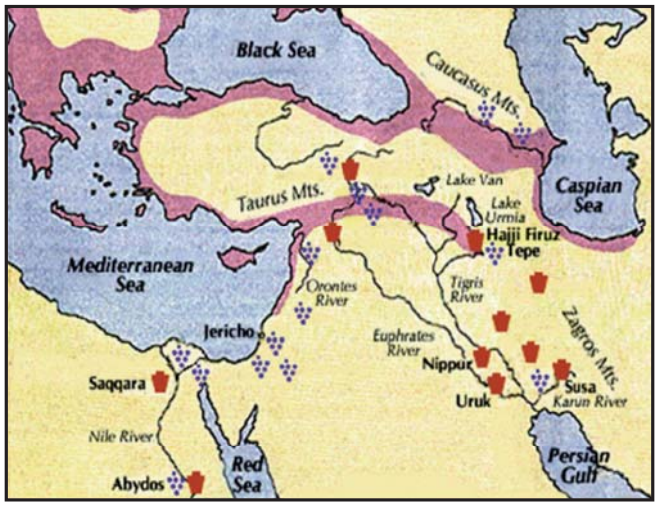

Figure 1. above
Map of McGovern (2003)

Figure 2. left
Seeds of grape, Dangreuli gora, VI-V mil. BC.

Figure 3. right
Location of archaeological sites near Tbilisi.

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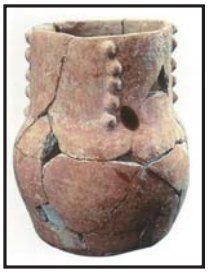

Left to right
Figure 4. Vessel from Arukhlo, IV mil. BC.

Figure 5. Fragment of vessel with grape relief, Khramis didi gora, VI-V mil. BC.

Figure 6. Goddesses of Fertility, Khramis didi gora, VI-V mil. BC.

of grapevine and others). Among these findings seeds of grapevine are more important for us. Based on of their morphological and ampelometric characteristic and comparison to ampelographic parameters of modern varieties of grapevine, was made conclusion, that seeds from *Shulaveri* bring nearest of cultural grapevine *V. vinifera* L. ssp. *sativa* D.C. Another finding from the same site are: wine vessel '*Dergi'* with relief of grape bunch, friable fragments of other thick-walled vessels with relief representation of grape grains, statues of a Goddesses of Fertility others (Chilashvili, 2004). (Figg. 4, 5 and 6). Patrick Mc. Govern (2003b) from the University of Pennsylvania Museum reported about discovery of wine residues from vine jar from *Shulaveri*.

Figure 7. N. Vavilov (1931)

Wild grapevine in nature

Based on result of investigation wild and cultivated grapevine of the South Caucasus N. Vavilov (1931) (Fig. 7) - the author of the centres of domestication of cultivated plants - concludes: "*All existing data indicates that the Caucasus is the main hearth of origin of wild and cultivated grapevine. Great number of various autochthonous varieties in Georgia, Azerbaijan*

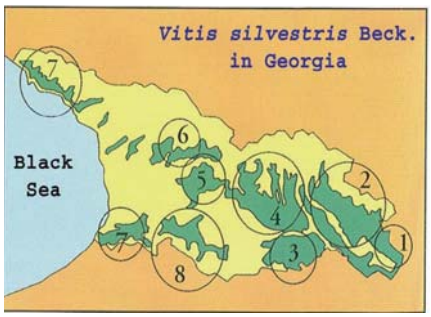

Figure 8. Map of the Wild grapevine spreads in the 8 main centers of Georgia in XX century (Del Zan et al. 2004, 2009).

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Left to right
Figure 9, Figure 10.
Two examples of plants of different high and age.

and Armenia, which have striking diversity of colour and shape of berries and seeds, indicate about concentration the processes of form origin here".

The Wild grapevine was spread in the 8 main centers of Georgia in XX century (Del Zan *et al*. 2004, 2009) (Fig. 8).

Plants of wild grape in nature has different dimension, depending on age of plants and conditions of living area (two examples of plants of different high and age in Figures 9 and 10).

A wild grapevine is leaving in various location, but prefer river gorges with higher humidity and alluvial soils (two examples of locations in Figures 11 and 12).

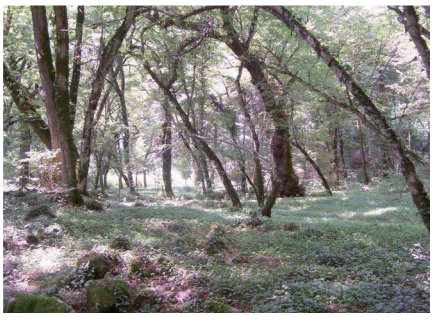

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Figure 11, Figure 12.
Two examples of locations.

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Characterizations and conservation of wild grapevine

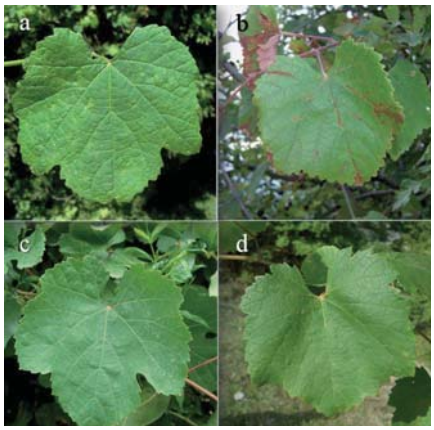

Figure 13. Leaves belonging to four plants from different location: a) Bagichal; b) Ikalto; c) Nakhidu; d) Kvetari.

In wild grapevine there is a variability in the morphology of mature leaves from one plant to another. As examples, the pictures show leaves belonging to four plants from a different location:

a) Bagichala; b) Ikalto; c) Nakhidu; d) Kvetari.

Leaves are one of the elements that must be analyzed during ampelographic characterization. Indeed, this analysis takes into account every part of plant: young and mature shoots, young and mature leaves, inflorescences, bunch, berry and seeds (OIV, 1983). The observation are conducted in the different phenological stages of the plant.

In the study of the grapevine this analysis allows a phenotypic description only. To better appreciate grapevine biodiversity genetic characterization must be carried out. This analysis uses genetic marker well known in literature, the most utilized are the Simple Sequence Repeats (SSR) or Microsatellites. These consist in brief genoma sequences (dinucleotids, trinucleotids or tetranucleotids) repeated inside a not-coding DNA region, with size range from 50 bases to 350.

Figure 14, Figure 15. Activities for re-covering of forms collected yearly by R. Ramishvili within the country.

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Considering the close link with the domestic grapevine and its high biodiversity in Georgia, it is important to preserve the wild germplasm, because its natural habitat are threatened by human activities. Propagation of wild grapevine from Georgia has been done at the University of Milan (Figure 14). The operation includes activities for re-covering of forms collected yearly by R. Ramishvili within the country (Figure 15).

Materials and Methods

Exploration method was used for investigation of wild vine in Georgia. Discovered plants have been described on places of their location by Global Positioning System (GPS) and the FAO-IPGRI Multicrop Passport Descriptors have been completed. Photos and herbarium have been done for further investigation of plants.

Harmonized ampelographic descriptors of OIV (1983, 2007), IPGRI (1997) and GENRES 081 were used for ampelographic, agronomic and cytological characterization of vine organs. Eighty one parameters of leaf were obtained by the software "SuperAmpelo" (Soldavini *et al.,* 2007). Grape parameters of female plants as well pollen parameters for male plants were studied. Anthocyanin analysis was made by HPLC technique.

In 2008 a joint expedition was organized in collaboration of the University of Sevilla and the Institute of Horticulture, Viticulture and Oenology, Tbilisi for investigation of sanitary status of wild grapevine *in-situ* populations (Maghradze *et al*., 2010b). The most 10 representative population was investigated. In addition accompaniment to wild vine flora was described.

Particular target of this work was a description of the Georgian germplasm platform through a molecular approach based on SSR profiling with representatives of wild populations and most interesting wine/table cultivated grapes of Georgia, including 22 genotypes of wild vine and 139 varieties. The germplasm was characterized by 20 SSR markers, located in 19 chromosomes. Collection of samples for DNA

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extraction, PCR, amplification, selection SSR markers and characterization of obtained molecular data have been done by similar to cultivated varieties way as it was described before (Maghradze *et. al.*, 2009a, Maghradze *et. al.*, 2010a).

For propagation of vine samples were used winter woody canes, collected in natural habitations of Georgia. For recovering of Ramishvili's accessions woody canes were collected in old Dighomi collection, Georgia. The canes were rooted in a special substrate containing perlite in it. Gibberellin's acid was used for stimulation of rooting process. The cuttings were situated in controlled air conditions of a greenhouse. The new-obtained plants with well-developed roots were transferred to plots with fertile soil for further growing and maintenance. The plots were placed in open area.

Diverse methods of uni- and multivariate statistical analyses based on the SSPS software were used for calculation of obtained data.

Results and discussion

Fifty populations of wild grapevine with 189 plants were described on the territory of the country for this period. Size of population is diverse and varies from 1 to 20 plants, showing average quantity of 3.8 plants per site (Table 1). According to number of plants in the populations they has been evaluated mostly as 'Very bad' (64%) or 'Bad' (24%) and just only 12% - as 'Regular' (Figure 16).

Table 1. Recent status of wild populations in nature.

No of sites	Total nr. of plants	Plant per site	range of plant No
50	189	3,8	1 - 20

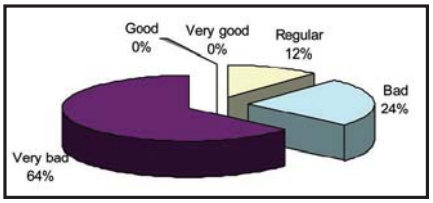

Figure 16. Status of populations.

Sex structure of all populations was one of the first characters under observation as one of important character (among others) allowing to discriminate among wild and cultivated grapevine.

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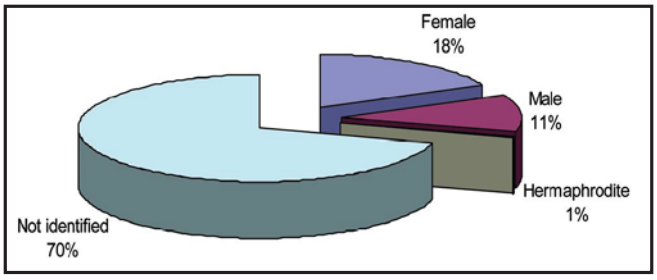

Figure 17.
Sex segregation of discovered wild plants.

Observing flowers in nature is sometime not easy because of the short flowering time duration and the position of the flowers, often high in the canopy (Figure 17). In spite of this, for several population we have complete data: the population 'Shirikhevi' with 8 plants contains 3 female and 5 male plants; the population 'Misaktsieli' with 5 plants contains 5 female and 0 mail plants; 2-2 female plants were described in the populations 'Barisakho Turning' and 'Ikalto' containing 2-2 plants in each. These examples indicates that, if the plants in nature are males or females still today, and no hermaphrodites are found in the population, the gene flow from the cultivated area was small and probably most of the hybrid progenies did not survive – (otherwise we should find an increasing number of hermaphrodites in nature, Di Vecchi *et al*, 2008; Terral *et al,* 2009).

Sex characterisation was also carried out on Georgian autochthonous varieties. A result was that 55 varieties among 414 ones described in the Ampelography of the Soviet Union (1949-1970) are females (13.3%), while the remaining ones are hermaphrodites (Maghradze *et al.*, 2010b). And in our collection of Georgian local varieties, located in Gorizia (Italy), 18.0% of varieties have female flower among available 150 ones. Such an high number of survived female varieties in the Georgian germplasm (while in the whole European cultivated grapevine gene pool the percent of female is below 8%), gives one additional hint about the domestication process of grapevine having directly interested the territory of this country.

Most number of discovered plants was founded in places closed rivers' banks in humid areas. Only several exemplars where founded in forests near from water resources. Higher point of discovered wild grapevine was 980 m above sea level (village Meneso, Dusheti district). The wild vine uses diverse trees for support: hawthorn and *Prunus divaricata* (mainly), also asp, willow, cornel, hornbeam and other.

Most of founded plants have characteristics as follow: tip of young

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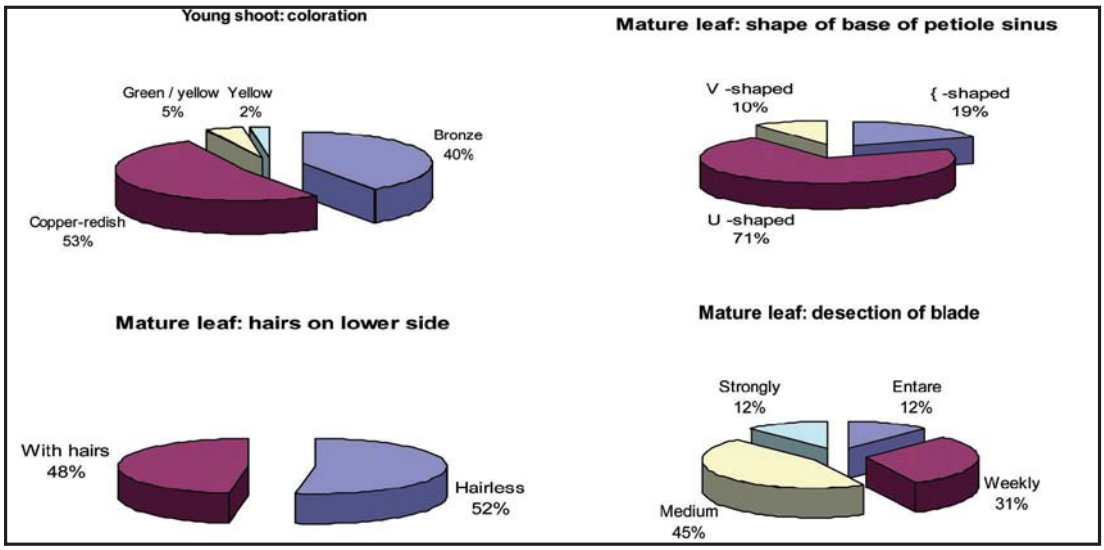
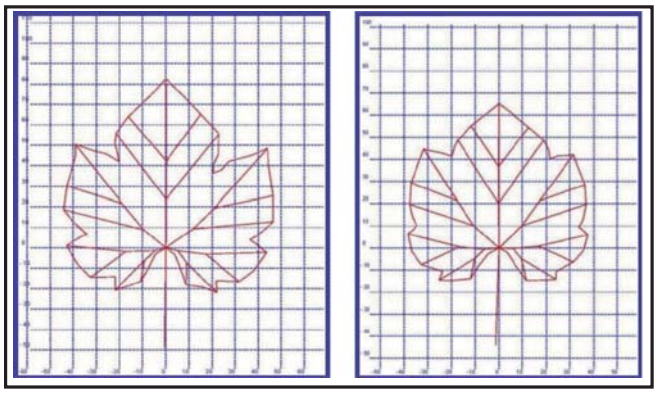


Figure 18. Variability of ampelographic parameters.

shoot is covered by strong hears; shapes of matured leaves are ovate or rounded, has small or medium size and weak hears on a lower leaf side; length of petiole is less than the length of the main vein of a leaf; petiole sinus is open; the inflorescences are male and female; bunch is small and loose. Berry is small in size, mainly round and black in colour.

Forty two rooted genotypes were studded according to 15 characteristics and correspondent descriptors of the OIV, IPGRI and GENRES have been completed for young shoot, young leaf and mature leaf (Figure 18, 19) in 2009, demonstrating variability of these parameters.

Figure 19. Images of leaves taken by SuperAmpelo. i) Archveti 01; ii) Baga 01



Grape of 11 wild forms were collected from various location of *in-situ* conditions in 2008. They were characterised by 11 traits of bunch, berry and must. Experimental wines of two wild accessions were made and analysed by HPLC technique (Table 2). Pollen parameters (length, width and diameter) of 7 wild exemplars were investigated.

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Accession name: Wild vine "Samebis seri 09"
Year of harvest: 2008
Year of wine studding: 2009
1. Titratable acidity (g/l).....9,9
2. pH 3,6
3. Total phenols (g/l).....8,74
4. Tannins (g/l) 9,2
5. Massive concentration of colorants (mg/l).....359,28 (norm is 30-500 mg/l)
6. Organic acids (g/l):
   - Tartaric acid.....1,48
   - Malic acid 0
7. Antocyanins (mg/l):
   - Total.....
   - Kuromanin chloride.....5,315
   - Delphinidin chloride 1,455
   - Peonidin - 3 - glucoside chloride.....12,134
   - Malvidin chloride 18,73
8. Malvidin Diglucoside (mg/l).....0

Table 2. Chemical analyses of wine /Laboratory of the IHVO/

The joint Spanish-Georgian expedition for investigation of sanitary status of wild grapevine *in-situ* populations reported about evaluation of the main pests and fungal diseases of wild plants: i) Root form of *Phylloxera* was not detected, while gals are evident on leaves of root-stocks in Georgia; ii) Among Mites (Acari, Eriophyidae) *Colomerus vitis* (Pagenstecher) was abundant, while *Calepitrimerus vitis* (Nalepa) was observed on half of plants; iii) The fungal diseases Powdery mildew and Downy mildew have been detected in all observed populations.

Accompaniment flora to wild vine in natural ecosystems is mostly following: *Acer campestris, Carpinus caucasica, Carpinus orientalis, Celtis caucasica, Celtis australis, Cichorium intybus, Clematis vitalba, Cornus mas, Cornus australis, Corylus avellana, Cotinus coggygria, Crataegus monogyna, Berberis vulgaris, Berberis iberica, Diospyrus lotus, Fagus orientalis, Ficus carica, Fraxinus sp., Hedera helix, Hedera caucasigen, Hippophae rhamnoides, Juglans regia, Ligustrum vulgare, Malus orientalis, Mespilus germanica, Morus sp., Origanum vulgare, Paliurus spina-christi, Platanus, Populus tremula, Prunus avium, Prunus divaricata, Pyrus caucasica, Punica granatum, Rosa canina, Rubus sp, Sambucus ebulus, Salix sp., Smilax aspera, Quercus sp., Ulmus sp.* and others.

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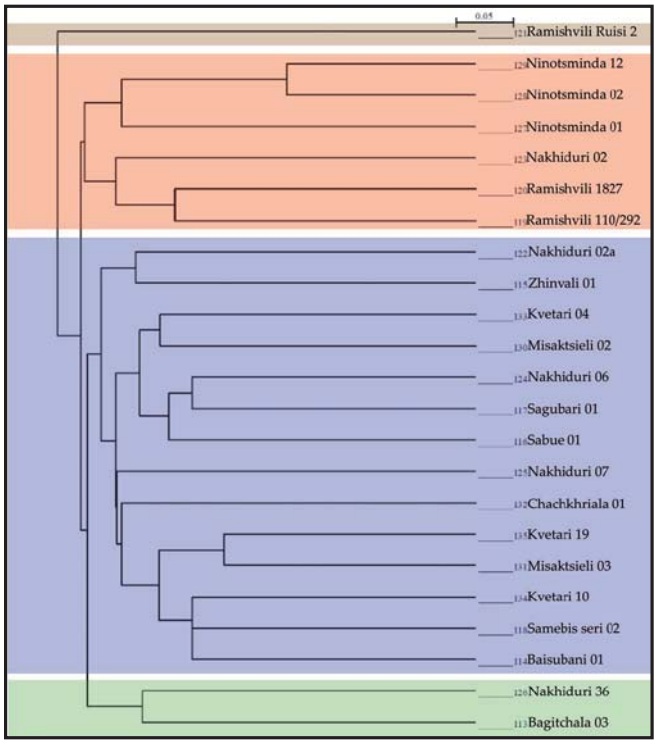

Figure 20. Clustering of wild vines by 20 SSR markers.

The molecular fingerprint has revealed that wild accessions are well distinguished from the cultivated compartments as already seen in the viticulture of other European Regions, even if in this case the separation seems not to be as great as the one identified in other countries (Figure 20); very few mistakes of taxon attribution (*sativa* instead of *sylvestris*) or sub-specific intercrossings (*sativa x sylvestris*, the plan being an hybrid instead of a pure *sylvestris*) were detected in the wild samples; in the same way, no cases of parent-offspring relationship among wild and cultivated accessions have been detected. Four main groups were detected inside of wild gene pool of Georgia.

Only a few times ago so widespread plant was so much reduced, that it is included in the "Red Book of Georgia" (1982) for *in-situ* preservation recently. The fact of fast genetic erosion become clear during our re-inventory of wild plants discovered by R. Ramishvili in previous years, while we could find only two plants in Meneso location and it seams that other plants were eliminated from natural environment. Even if just only partial ethnographic-linguist research demonstrated, that the Georgian language and its dialects have various names for wild grapevine like '*Krikina'*, '*Dzghuambli/Zghvambli*', '*Usurvazi*', '*Burdzghuami*', '*Mortskhula*', '*Burekhi*', '*Shchurishi*' (Javakhishvili, 1934), '*Omtskhvaro*', '*Mtskhero*', '*Tkis Vazi/Kurdzeni*', '*Datvkurdzena*' (Pruidze, 1966), '*Babilo*' and some others, indicating about presence of this plant in different historical provinces of Georgia (in spite of modern restricted spreading) since long historical period. Reduction takes

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place due to attack of pathogens and influence of a men on the environment.

Regarding conservation just several forms are in *ex-situ* preservation in Telavi collection and Tbilisi Botanical Garden. Just populations available in the reserve areas of Georgia are conserved. In spite of this fact no one specific program for conservation works in the country, like not available any target program for inventory, description and investigation of wild grapevine in Georgia.

Forty-six genotypes of wild grapevine were rooted in a greenhouse in Italy and 77 plants were obtained at the University of Milan. The 7 genotypes of vine, collected by R. Ramishvili in the end of XX century, were also recovered in the same greenhouse.

Conclusion

Wild grapevine *V. sylvestris* Gmel. still occurs on the territory of Georgia. The molecular fingerprint has demonstrated that wild accessions are well distinguished from the cultivated compartments. But it is now a rare plant. The size of the populations has today a tendency to decrease, both due to disease attacks and to the increased use of the land by agriculture and other human activities.

Nevertheless we believe these populations still bears a high value for viticulture, as a reservoir of interesting genes; for example, since the actual living wild grapevine plants still resist *Phylloxera* attacks in nature, they may host resistance genetic mechanisms interesting to explore. Present initiatives for preservation are often not sufficient (*ex-situ*) or not implemented (*in-situ*), and future conservation and investigation activities are required.

Acknowledgments

To: the international project of Bioversity International “Conservation and use grapevine genetic resources in the Caucasus and Northern Black

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Sea region" (2003-2008); "ECO-NET" project of the Ministry of Foreign Affairs of France (2006-2007); EU project "GrapeGen06: Conservation, characterization and management of grapevine genetic resources in Europe" (2008–2010); Dr. Josef Turok (CGIAR-Tashkent); Dr. Valerie Laucou (INRA – Montpellier, France).

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Chapter 4

Ecology and Conservation

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Support trees and shrubs for the Eurasian wild grapevine in Southern Caucasus

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ABSTRACT

A prospecting of habitats and mechanical support host species for the climber Eurasian wild grapevine, *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi, was carried out on 13 natural populations situated along river bank forests, floodplains and colluvial positions in Georgia (Marneuli, Mtskheta and Gori districts, Gardabani Protected area and Lagodekhi Reserve), Armenia (Akhtala and Tavoush regions) and Azerbaijan (Quba region) during survey of 2013. The research demonstrated that Eurasian wild grapevine (*Vitis vinifera* subsp. *sylvestris*) is found in Southern Caucasus in a wide variety of habitats always linked to water availability. *Punica granatum* trees are the commonest mechanical support for wild grapevine in the South Caucasus and *Hedera helix* often shares the same support trees. However we documented wild grapevines climbing on other 24 different species of trees and large shrubs and, further, 32 associated species. We determined, four different clusters of localities using Structure software and the Weighted Neighbor Joining tree. These clusters are characterized by specific mechanical support and accompanying species. Other vines competing for host with Eurasian wild grapevine belong to the genera *Clematis, Hedera, Humulus, Smilax* and *Vitis* ssp.

Introduction

Wild grapevine (*Vitis vinifera* subsp. *sylvestris* (C.C.Gmel.) Hegi) is a tendril-bearer, woody climber inhabiting forests and scrub along river banks and ravine beds, from Western Europe to Central Asia. It is also available in the South Caucasus area [1] where it is particularly scattered along low caudal watercourses. Zecca et al. [2] have found one Armenian wild grapevine specimen to be the oldest lineage of *V. vinifera* subsp. *sylvestris* among those included in their study, being the Caucasian lineage the result of a division between *Vitis vinifera* and the Asian lineages in the late Miocene. This is coherent with the results of Pipia et al. [3] studying plastidial DNA and confirms the relevance of the Caucasus wild grapevine populations in the evolution of wild (and cultivated) grapevine, as the cradle of the viticulture [4].

The study of wild grapevine in the Caucasus developed by our team led to de discovery of sanitary problems in roots and aerial parts [5,6].

As a vine, *V. vinifera* subsp. *sylvestris,* although woody, it cannot remain free-standing to any appreciable height. In order to climb, vines need to locate and somehow grasp, lean or hook onto suitable supports [7]. At present studies on lianas or vines and the host species providing

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Table 1
Studied wild grapevine populations in Georgia, Azerbaijan and Armenia in 2013.

Site name	District	River	Interval of latitude N	Interval of longitude E	Alt.	P*
Georgia						
Nakhiduri	Marneuli	Ktsia	41°29′26″ - 41°29′13″	44°40′ 51″ - 44°41′22″	445	C
Tsitsamuri	Mtskheta	Aragvi	41°52′28″ - 41°52′38″	44°43′51″ - 44°43′ 57	469	C
Tedotsminda	Gori	Liakhvi	42°2′4″- 42°2′20″	44°3′19″-44°3′42″	639	C
Gardabani	Gardabani	Mtkvari	41°22′10″- 41°22′19″	45°4′6,3″ - 45°4′37″	274	F
Skra	Gori	Mtkvari	41°59′11″ - 41°59′13″	44°2′47″ - 44°2′47″	609	C
Lagodekhi	Lagodekhi	Matmiskhevi	41°48′2″- 41°48′45″″	46°19′12 - 46°20′24″	501	A
Azerbaijan						
Guruchai-1	Quba	Guruchai	41°24′1″	48°26′37″	680	F
Guruchai-2	Quba	Guruchai	41°26′3″- 41°26′3″	48°33′ 41″ - 48°33′50″	404	F
Rostov road Qusarchai 1 & 2	Quba	Qusarchai	41°28′6″ - 41°28′9″	48°33′57″ - 48°33′59″	385	F
Dellekkend**	Quba	Guruchai	41°24′37″	48°35′13″	413	F
Ağbil**	Quba	Qusarchai	41°25′32″ - 41°25′35″	48°33′54″ - 48°34′4″	415	F
Armenia						
Akhtala	Akhtala	Debed	41°6′18,3″- 41°7′15,8″	44°42′23 - 44°45′16,3″	644	C
Getahovit	Tavoush	Getik	40°54′6″- 40°54′ 8,7″3″	45°7′5 - 45°7′ 9,6″	719	C

Codes: Alt. Altitude (masl). P* (Position): A: riverbank forest; C: colluvial position (slope of a hill); F: flood plain. ** Not included in the final analysis.

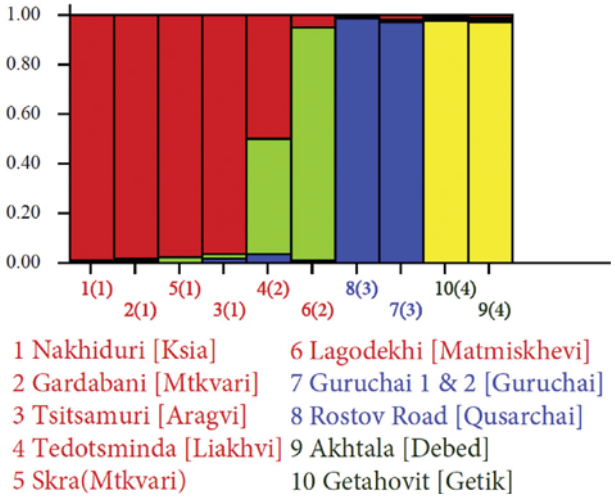

Fig. 1. Structure of the sampled populations in Southern Caucasu.
Note: Color codes: Bars: Red, group 1; Green, group 2; Blue, group 3; Yellow, Group 4; labels and countries: Red, Georgia; Blue, Azerbaijan; Green, Armenia.

their mechanical support are scarce although vines may exhibit host specificity based on the tree species identity, size or shape [8,9].

The objective of the present work is to analyze the species that provide mechanical support to the wild grapevine in Southern Caucasus, and the geographic structure of the ensemble.

Material and methods

The study of species associated to wild grapevine and characterizing the habitats was simultaneous to the sanitary prospection of natural populations of wild grape organized in Georgia, Armenia and Azerbaijan in October 2013 [6]. These zones are included within the Holarctic kingdom, Eurosiberian region, and assigned to the Caucasian or Irano-Turanian, biogeoghraphical provinces. The location based on GPS coordinates and the habitats of the different populations studied is shown in Table 1.

Sampling plots were irregular according, in each site, to the structure of wild grapevine populations. Trees were recorded as a host when the branches of the vine grew clearly supported on their branches and a part of the foliage of the vine appeared intermixed or above the one of the host.

Photographs and voucher specimens were collected for confirming in field preliminary identification of species. Identification process was conducted in the different institutes and universities of the authors and revised at the Plant Biology and Ecology Department of the Universidad de Seville (Spain) using as basic resources different floras of Armenia, Azerbaijan and Georgia [10–13].

Nomenclature of species and abbreviations of authors were standardized according to The Plant List [14].

Data were first organized in a 59 taxa x 10 localities matrix, where presence was coded as 1 for associated species and 10 for species further acting as mechanical support for Eurasian wild grapevine individuals. The transposed matrix was generated later. This matrix was processed using DARwin 6.0 [15]. Two dissimilarity matrices were calculated [16] for localities (Units: 10 and Variables: 59, Dissimilarity index: Counts - Chi^2, 500 bootstraps, this is an even dissimilarity which is a Euclidean distance) and species (Units: 59 and Variables: 10, Dissimilarity index: Counts - Chi^2, no bootstraps, this is an even dissimilarity which is a Euclidean distance). Weighted neighbor-joining tree was calculated for localities. A hierarchical tree for species was constructed using the Ward's minimum variance algorithm [16]. These trees were further processed with FigTree v1.4.3 [17]. We used Structure [18] which works using stochastic Bayesian methods of Markov Chains – Monte Carlo, and Harvester [19] in order to determine the optimal number of groups of localities. This last software set focus on molecular studies thus we adapted for Structure our data (species presence) in terms of haplotype alleles.

Results and discussion

Pomegranate (*Punica granatum*) is the commonest mechanical support species for Eurasian wild grapevine in the sites studied, and *Hedera helix* often shares the same support trees. However we documented wild grapevines climbing on other 24 different species of trees and large shrubs and, further, 32 associated species. We determined, four different clusters of localities using Structure (Fig. 1) and the Weighted Neighbor Joining tree (Fig. 2). These clusters are characterized by specific mechanical support and accompanying species (Fig. 3).

Wild grapevine-associated and mechanical support species follow primarily a major biogeographical pattern. Groups 1, 2 and 4 roughly fall within the Euro-Siberian Region and group 3 within the borders of the Irano-Turanian Region.

Group 1 is present in colluvial and flood plain forestall areas of central Georgia (Fig. 4a) within *Carpinus – Quercus* forests, at altitudes from 250 to 610 m above sea level. *Carpinus betulus*, *Cornus mas, Cornus sanguinea, Crataegus caucasica* and *C. monogyna*, *Diospyros lotus, Pyrus caucasica, Paliurus spina-christi, Populus alba* and *Corylus avellana* provide mechanical support to *Vitis vinifera* subsp. *sylvestris*. Other species of trees, like *Acer monspessulanum, Acer platanoides, Fagus orientalis* and

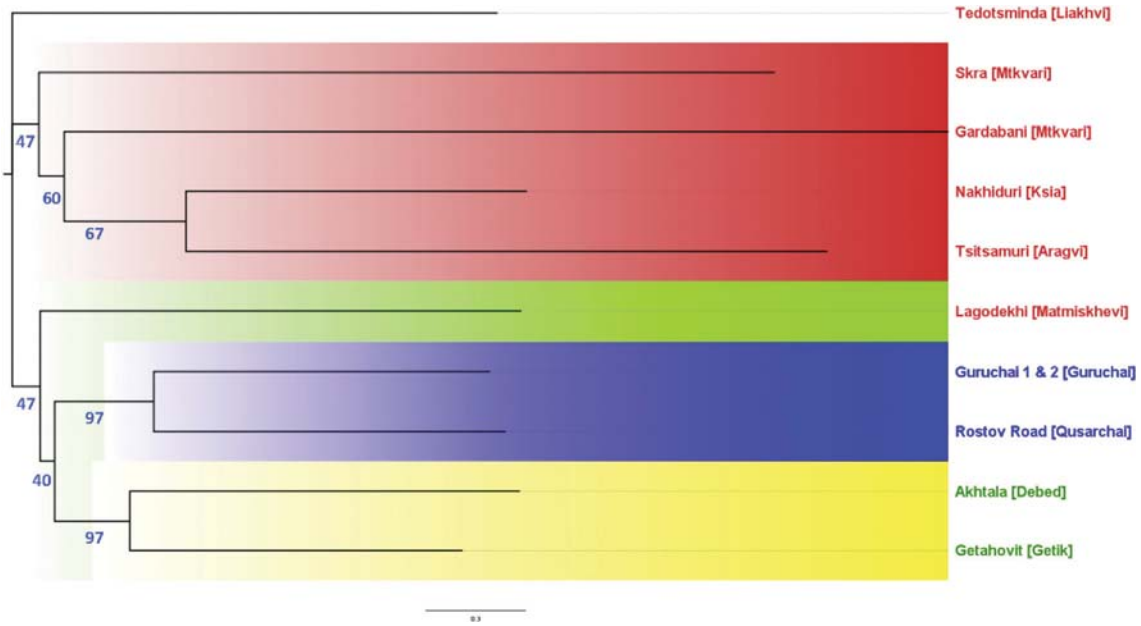

Fig. 2. Weighted Neighbor Joining tree for populations in Southern Caucasus.
Note: numbers below branches represent support in percentage of 500 bootstraps. Color codes: Shadows: Red, group 1; Green, group 2; Blue, group 3; Yellow, Group 4; tip labels: Red, Georgia; Blue, Azerbaijan; Green, Armenia.

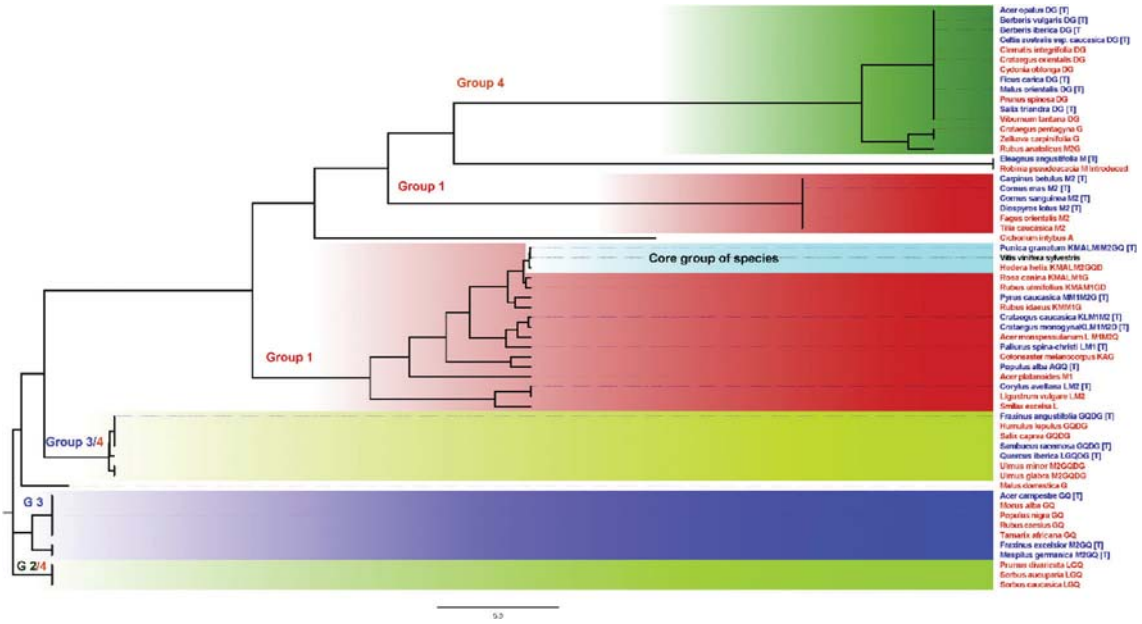

Fig. 3. Minimum variance hierarchical tree for species.
Note: labels above branches represent groups in Figs. 1 and 2. Color codes: labels: Red, species associated; Blue, species providing mechanical support.

Tilia caucasica were present but we did not recorded wild grapevine climbing on these. Accompanying species include shrubs like *Cotoneaster melanocarpus*, *Ligustrum vulgare, Rosa canina,* and brambles such as *Rubus ulmifolius, Rubus idaeus*.

Group 2 is present in the riverbank forest of Lagodekhi in eastern Georgia (Fig. 4b) at c. 500 m of altitude. Here *Corylus avellana, Prunus divaricata, Quercus iberica, Sorbus aucuparia* and *S. caucasica* are main mechanical supports. Other accompanying species include shrubs like *Ligustrum vulgare* and climbers such as *Smilax excelsa.*

Group 3 is present in wet flood plains of Irano-Turanian territories of eastern Azerbaijan (Fig. 4c). It shows a relatively high anthropic impact (*Morus alba*, *Populus nigra* plantations). Here *Fraxinus angustifolia, F. excelsior, Mespilus germanica*, *Prunus divaricata, Sambucus racemosa, Sorbus aucuparia* and *S. caucasica* are main mechanical supports. Other trees present are *Salix caprea, Ulmus glabra* and *U. minor*. Another climbers are *Humulus lupulus* and the invader species *Vitis rupestris* and *V. vulpina*, which are escaped rootstocks.

Group 4 is present in hillslopes of Armenia (Fig. 4d). Here trees and large shrubs grow sparse. Some, like *Acer hyrcanum, Berberis vulgaris* and *B. iberica, Celtis australis* subsp. *caucasica*, *Ficus carica, Malus orientalis, Fraxinus angustifolia*, *Quercus iberica*, *Sambucus racemosa* and *Salix triandra* act as main mechanical supports for wild grapevine. Other

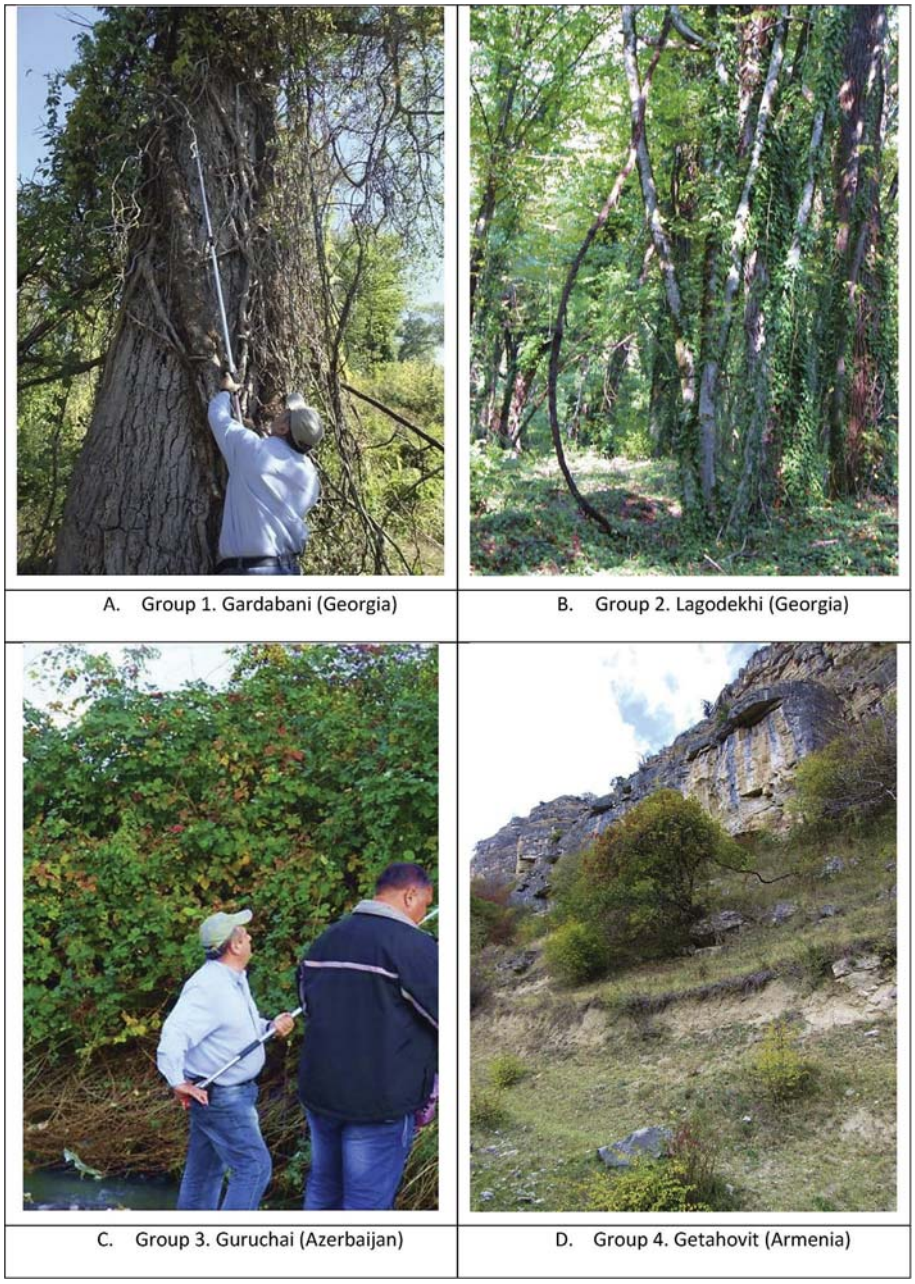

Fig. 4. Representative localities for Eurasian wild grapevine in the Caucasus.

trees and shrubs present are *Crataegus orientalis* and *C. pentagyna, Cydonia oblonga, Prunus spinosa, Salix caprea, Ulmus glabra* and *U. minor, Viburnum lantana* and *Zelkova carpinifolia.* Climbers and brambles include *Clematis vitalba, Humulus lupulus*, and *Rubus anatolicus.* Unlike dense forests, here the shoots of the vine often must progress at ground level several meters to find a tree on which they can grow successfully (Fig. 4D). Here, grapevines growing closely at the foot of the cliff take advantage of the rocks as a mechanical support in place of trees and shrubs (Fig. 5).

The presence in the sampled localities of tree and shrub species that do not support Eurasian wild grapevine lianas may be due to mechanical characteristics of the host, human intervention in the case of plantations (*Malus domestica, Morus alba, Populus nigra, Robinia pseudoacacia*) or simply at random. This is worthy of further investigation.

Conclusions

Eurasian wild grapevine (*Vitis vinifera* subsp. *sylvestris*) is found in Southern Caucasus in a wide variety of habitats always linked to water availability. In forests and scrubs wild grapevines require mechanical support provided by trees and large shrubs but also can climb on cliffs.

Mechanical support is often provided by *Punica granatum* and other numerous tree and large shrub species of the genera *Acer, Berberis, Crataegus, Diospyros, Elaeagnus, Ficus, Fraxinus, Malus, Mespilus, Populus, Paliurus, Pyrus, Quercus, Salix* and *Sambucus* that are more specific in habitat requirements.

Other vines competing for host with Eurasian wild grapevine belong to the genera *Clematis, Hedera, Humulus, Smilax* and *Vitis* ssp.

Fig. 5. Eurasian wild grapevine climbing the cliff in Getahovit (Armenia).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aasci.2018.06.005.

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Research Note

Micropropagation and *in vitro* germplasm conservation of Georgian wild grapevines

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Key words: tissue culture; South Caucasus; plant development.

Introduction: Wild grapevine *Vitis vinifera* ssp *sylvestris* Gmel., considered the wild ancestor of the cultivated grapevine *Vitis vinifera* ssp *sativa* D.C., is a typical representative of the Georgian flora (Maghradze *et al*. 2012). Fifty populations of wild grapevine have been described in this zone of the south of Caucasus. Population size varies from 1 to 20 plants, with an average of 3.8 plants per site (Chkhartishvili *et al*. 2005, Maghradze *et al*. 2006). It grows mainly in river gorges, the common habitat of wild grapevine. The lack of human selection has resulted in a highly conserved genetic diversity, so it can play an important role as plant genetic resource for further improvement of grapevine cultivars.

In vitro vegetative propagation of grapevine plants (micropropagation) has been used successfully by different authors (Cantos *et al*. 1993, Nicholson *et al*. 2012). These authors reported the development of a high number of plants starting with a few initial explants, in a relatively small space. *In vitro* growth is often strong due to rejuvenation, disease free, optimal nutrition balance and independence of the seasonal period. This technique is usually used for a suitable material conservation.

The objective of this research was testing the response of five Georgian wild grapevine accessions to micropropagation and comparing the resulting plants with other commercial and wild grapevines.

Material and Methods: Cuttings from five wild grapevine plants from two Georgian populations, Bagichala 10 (G10) and Tedotsminda 01 (G1), 06 (G6), 17 (G17) and 21 (G21) located in Aragvi river basin at Dusheti district and in Liakhvi river basin at Gori district correspondently, Kartli Province, East Georgia were taken, base-dipped in a solution with 2.5 % sucrose and 0.6 % of the fungicide benomyl and placed in a growth chamber with 23 ± 2 °C, 111 µmol·m^{-2}·s^{-1} light intensity and 16 h photoperiod. From these cuttings, sprouting shoots with 3-4 buds, 1.5 cm long were chosen and disinfected by the following steps: (a) immersion in 70 % ethanol for 1 min; (b) immersion in 12 % sodium hypochlorite (10 % active chlorine) with some drops of Tween-20, for 12 min; and (c) rinsing with sterilized water (three times, 5 min. each time).

After disinfection, uninodal explants (0.3-0.5 cm long) with one bud, were placed separately in sterile test tubes (25 x 150 mm) with 8 mL of culture medium (Sarmiento *et al*. 1992), modified with 2.5 % sucrose, 0.072 mg·L^{-1} of BAP, 0.024 mg·L^{-1} of NAA and 0.6 % agar, pH 5.7. Each tube was covered with a plastic cap, sealed with parafilm and placed in a growth chamber at 23 ± 2 °C, 30 µmol·m^{-2} s^{-1} and 16 h photoperiod. Explants from rootstock varieties 'Ramsey', '110-Richter' (110R), '161-49 Couderc' (161-49), '41B' and 'CH' (from a saline semi-arid zone in Arica-Chile); from *Vitis vinifera* varieties 'Superior Seedless' (SS), 'Malvasia' and 'Pedro Ximénez' (PX), and from the Andalusian wild grapevines 'Serag', '14/Córdoba/3' (CO3); '14/Rute/1' (CO9); '14/Montoro/4' (CO8); '14/Montoro/3' (CO7) and '23/Guarromán/2' (J3) (Ocete *et al.* 2007), were obtained from the *in vitro* germplasm bank of IRNAS-CSIC. Similar uninodal explants (Table) from these accessions were cultured in the same micropropagation conditions described above. After 60 d of *in vitro* culture the number of surviving plants, plant size (length, bud number per shoot and axillary shoot number) and root development (percentage of rooting and root number per plant) were determined.

For acclimatization, 10 rooted plants from each Georgian wild grapevine obtained in the micropropagation process were adapted to *ex vitro* conditions according to Cantos *et al.* (1998).

Statistical analysis was carried out using IBM SPSS Statistics v. 22. Data were analysed using ANOVA. Tukey test was applied for identification of important contrasts. Differences in percentages cases were compared using z test.

Results and Discussion: The average survival of all tested accessions was 78.5 % (Table). All the Georgian accessions, except G6 with a survival of 61 %, were above this value particularly G10 (100 %) and G17 (97.4 %). The three Georgian wild grapevines with higher survival ability were statistically higher than J3, 41B, PX and G6. The average stem length was 2.1 cm. This value is smaller ($p \leq 0.05$) than the length recorded for the Georgian accessions G10 (2.9 cm) and G17 (2.8 cm). These values are much lower than for 110R (4.5 cm) and CH (7.1 cm). The average number of buds per shoot for investigated accessions was 3.5 and the shoot number was 0.5, but the maximum values of these traits were detected in the plants belonging to CH (11.4 and 1.9 respectively), much higher than in other accessions ($p \leq 0.05$). The response of Georgian wild grapevines G10 and G17 was rather high, the average number of buds per shoot was 4.5 and the shoot number was 0.5 for the first sample and 5.4 and 1.0 for the second one which was significantly different in comparison with the same characteristics for the plants belonging to CH.

Explants of all accessions developed root system in the same micropropagation medium without any modification of phytoregulators. This behavior of grapevine explants with this medium is well known (Troncoso *et al*., 1990; Troncoso *et al.*, 1999). The average rooting percentage in our experiment was 70.2 %, less than that reported by Troncoso *et al*. (1999) (89.7 %). Georgian wild grapevines G10, G17 and G21 presented very high percentages of rooted plants, 87.5, 94.7 and 89.7 % respectively. G1 explants re-

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Table

Comparison among the average values of parameters obtained in the micropropagation of Georgian wild grapevine accessions (in grey colour) and the other considered accessions

Accession	Number of explants	Survival (%)	Stem length (cm)	Buds number per shoots	Shoots number	Rooting (%)	Roots number
G10	8	100 e*	2.9 c	4.5 def	0.5 abcd	87.5 f	1.6 a
G17	29	97.4 e	2.8 c	5.3 ef	1.00 cd	94.7 f	2.6 ab
Ramsey	53	96.3 e	1.3 abc	2.3 abcd	0.1 a	94.3 ef	2.9 ab
G21	57	93.2 de	1.7 abc	3.6 bcdef	0.4 abc	89.7 def	2.3 ab
G1	33	91.4 bcde	1.8 abc	3.0 abcde	0.1 ab	82.9 bcdef	2.2 ab
110R	56	90.3 bcde	4.5 d	5.9 f	1.0 cd	75.8 bcdef	2.7 ab
Serag	77	89.4 bcde	1.9 abc	4.5 def	0.7 bcd	77.4 bcdef	3.5 ab
CH	39	88.4 bcde	7.1 e	11.4 g	1.9 e	74.4 bcdef	2.6 ab
SS	122	83.6 bcde	0.3 a	0.8 a	0.1 a	82.8 cdef	5.7 c
CO3	36	78.6 bcde	1.3 abc	3.1 abcde	0.5 abcd	69.0 abcdef	2.4 ab
CO9	44	77.8 bcde	0.9 ab	1.4 ab	0.2 ab	71.1 bcdef	3.7 abc
161-49	24	77.8 abcde	1.5 abc	2.9 abcde	0.1 ab	59.3 abcdef	2.9 ab
Malvasía	67	74.7 bcde	1.4 abc	3.2 abcde	0.3 ab	51.9 ab	2.5 ab
CO8	42	68.8 abcd	1.3 abc	1.6 abc	0.1 ab	51.6 ab	2.9 ab
CO7	43	64.9 abcd	2.3 bc	3.4 bcdef	0.4 abc	64.9 abcd	3.6 abc
J3	42	61.8 a	1.1 ab	2.2 abcd	0.4 ab	56.4 ab	2.6 ab
G6	28	61 ab	1.4 abc	2.4 abcd	0.2 ab	56.1 abce	2.0 a
PX	30	56.9 ab	2.2 bc	4.2 cdef	1.07 d	56.9 ab	4.4 bc
41B	37	40 a	1.3 abc	1.5 ab	0.2 ab	36.7 a	3.7 abc
Average		78.5	2.1	3.5	0.5	70.2	2.9

* In each column, means followed by the same letter are not statistically different ($p = 0.05$).

duced this percentage to 82.9 and the lowest rooting was observed for G6, only 56.1 % with the trend described for the aerial part. The average number of roots was 2.99 for the plants of all 19 accessions. Plants of the five Georgian wild grapevines presented a lower number of roots, in a range between 2.6 (G17) and 1.6 (G10). These values were particularly low ($p \leq 0.05$) when compared to plants of Superior Seedless, 5.8.

Bud number per shoot was significantly correlated ($p < 0.0001$) with stem length ($r = 0.96$, $p < 0.0001$) for all accessions, including Georgian wild grapes. Other relationships detected were between shoot number and stem length ($r = 0.88$, $p < 0.0001$) and between shoot number and bud number per shoot ($r = 0.92$, $p < 0.0001$). There was also a significant correlation between survival and rooting ($r = 0.91$, $p < 0.0001$). On the other hand root number did not correlate with survival or rooting. These results demonstrated that the rooting was a more important parameter than root number for successful explant culture.

The Georgian plants showed in all cases a 100 % adaptation from *in vitro* to *ex vitro* conditions, indicating the suitable quality of the root system obtained by micropropagation.

Conclusion: In conclusion, we observed a good response of the considered Georgian wild grapevines to micropropagation and adaptation, surpassing other wild grapevines and cultivars with great importance in viticulture. In consequence, *in vitro* culture can be an appropriate conservation system for this Georgian material.

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Chapter 5

Genetic Diversity and Cultivar Linkage

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OPINION PAPER

From the cradle of grapevine domestication: molecular overview and description of Georgian grapevine (*Vitis vinifera* L.) germplasm

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Abstract Historical information and archaeological and palaeobotanical findings point Georgia, in the South Caucasus, as a cradle for grapevine (*Vitis vinifera* L.) domestication from its wild form (*V. vinifera silvestris* Beck.) and subsequent selection and development of varieties with characters suitable for human consumption. The hypothesis of Georgia being a center of domestication, combined with its distance from western countries and the importance of its viticulture and wine production, make Georgian grape germplasm particularly interesting to be investigated under the genetic point of view. Twenty nuclear microsatellite loci were used to genotype 112 Georgian grapevine accessions (*V. vinifera sativa* Beck.) from germplasm collections and 18 from spontaneous growing plants (*V. vinifera silvestris* Beck.) found in wild conditions and to compare them to a large international cultivar collection in France. Data analysis shows that Georgian grapevine germplasm has maintained distinctive traits despite arrival of international, foreign varieties and still conserve characteristics of local breeding linked to traditional wine production regions of the country. Results have identified alleles, overall loci, well represented in the Georgian germplasm (cultivated and wild) and absent or poorly represented in other countries, highlighting uniqueness and originality of traits of this viticulture. Moreover, the search for relationships between Georgian and foreign viticulture has evidenced few interesting cases linking the Georgian varieties with Western European ones and with neighboring Caucasian countries, helping to identify the real place of origin in some doubtful cases. In addition, populations or sparse individuals of wild grapevine still preserved in the Georgian natural environments present smaller genetic distances with local cultivars than in other European regions. Principal component analysis (PCA) has also identified special overlapping of the wild compartment with some cultivated varieties. This work provides a highly significant new contribution to applied aspects of Georgian grapevine genetic resources management and use. Uniqueness of the Georgian cultivated grapevine gene pool together with its close relatedness with the wild compartment makes this country a good candidate to address questions regarding domestication and grapevine genetic resource conservation.

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Introduction

Grapevine (*Vitis vinifera* L.) comprises cultivated (*V. vinifera* subsp. *sativa* Beck.) and wild forms (*V. vinifera* subsp. *silvestris* Beck.) originally dispersed from western Asia to Europe (Zohary and Horf 2000). Nowadays, more than 6,000 accessions are recorded as individual varieties (Alleweldt and Possingham 1988). The origin of most of them is still questionable or unknown due to (1) existence of several putative domestication centers, dispersed in all the distribution area of the wild progenitor; (2) exchange of plant material among countries; and (3) possible crossing among locally domesticated varieties and grapes imported from abroad.

The South Caucasus (Azerbaijan, Armenia, and Georgia), together with eastern Anatolia, has been considered for a long time as the birth place for viticulture with the earliest examples of winemaking (This et al. 2006; McGovern 2003a, b; Zohary and Horf 2000; Olmo 1995; Levadoux 1956; Negrul 1938; Vavilov 1926). Georgia is considered a cradle for the origin and domestication of cultivated grapevine *V. vinifera* L. subsp. *sativa* Beck., since many archaeological findings of this region are linked with viticultural and winemaking activities. Historical, ethnographical, religious, and toponimical information give additional argumentations supporting this theory (Hehn 1870; De Candolle 1883; Vavilov 1931; Kighuradze 2000; Ramishvili 2001; McGovern 2003a; Chilashvili 2004; Costantini et al. 2005/2006; Chkhartishvili and Maghradze 2012; Forni 2012; Forni and Failla 2010).

Archaeobotanical and archaeological data, dating back to the sixth and fifth millennium BC (Di Pasquale 2010; Rusishvili 2010; Licheli 2007), support evidence of presence of so-called Shulaveri-Shomu tepe culture (sixth to fourth millennium BC) in the Lower (Kvemo) Katli province of Georgia (sites of Shulaveri, Arukhlo, Khramis Didi Gora, Tsiteli Sopeli, and Kachaghana). Morphological and morphometric characteristics of grape seed remains collected from the site of Shulaveri village (McGovern 2003b) and dated in the sixth to the fifth millennium BC are close to modern cultivated grapevine (Rusishvili 2010; Kokrashvili 2004; Ramishvili 2001). The South Caucasus was covered by the Mtkvari (Kura)-Araks culture from the fourth to the second millennium BC. During the Bronze Age (fourth millennium BC), farming in the site of Badaani (Tianeti district of Kartli province) is witnessed by remains of common wheat (*Triticum aestivum* L. em Thell), Persian wheat (*Triticum carthlicum* Nev.), multirowed barley, and grapevine seeds (Japaridze and Javakhishvili 1971; Rushishvili 1990; Rusishvili 2010). Other grape seeds were discovered in a settlement of Kvatskhelebi, dating back to 2800 BC (Licheli 2007; Rusishvili 2010).

The Trialeti culture has evolved in the first part of the second millennium BC and reached its zenith around 1500 BC in Eastern Georgia, evidencing close ties with the highly developed cultures of the ancient world. According to Herodotus (fifth century BC) and Strabon (first century BC), winemaking prospered in Georgia. As also witnessed by: vine stems from Nosiri (Senaki district, second part of the second millennium BC), seeds of grapevine from Ergeta (Zugdidi district, seventh to sixth centuries BC), and Gienos (Ochamchire district, seventh to sixth centuries BC), belonging to both subspecies *sativa* and *silvestris* (Rushishvili 1990; Rusishvili 2010) as well as grape seeds from Anaklia (Zugdidi district) and Sokhumi (Dzidziguri 1995).

Viticulture and winemaking development continued during the Christian epoch (in Georgia since the fourth century AD). Archaeological findings of many wineries, with wine jars named Kvevri (Kvevri 2011 http://kvevri.org/; Glonti 2010), crushers named Satsnakheli, and other agricultural tools for winemaking and wine care were excavated as well as irrigation systems, terraces for grapevine cultivation, and as reported for previous periods, archaeological seed remains.

Grapevine plants became one of the main ornaments for Christian churches in Georgia. During the Middle Ages, grapevine was considered as a leading crop and winemaking was one of the most important activities (Licheli 2007).

Recent history

Georgian viticulture and winemaking benefited of the strict link with the development of capitalism in Russia, and new wineries, spirit, and brandy factories were constructed since the second part of the nineteenth century to meet the market demand. In 1879, the surface dedicated to vineyard reached 70,309 ha.

During the Soviet period, viticulture and winemaking were leading fields in Georgian agricultural activities and in 1973, the vineyard surface was enlarged up to 134,300 ha. Five Georgian local wine cultivars named Rkatsiteli, Saperavi, Tavkveri, Chinuri, and Kakhuri Mtsvane covered 42.7 % of vineyards of the former Soviet Union according to the 1985 census.

Ampelographical platform

It is not so easy to determine the exact number of autochthonous varieties (both table and wine) for this country. Ampelography of Georgia (Ketskhoveli et al. 1960) reports 525 autochthonous varieties with basic information, while the Ampelography of the Soviet Union (1949–1970) reports ampelographic description of 414 Georgian native varieties. These works together with other local ampelographic books are particularly useful, providing a morphological

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description of the plants and also defining, for the most important Georgian grape varieties, the old putative regions of origin, such as Kakheti, Kartli, Imereti, Racha, Lechkhumi, Samegrelo, Guria, Adjara, Abkhazeti, Saingilo, and Meskheti. At present, the main cultivated varieties in Georgia are autochthonous varieties having high-market value (Census 2004), while the best varieties like Rkatsiteli, Saperavi, Tavkveri, and Mtsvane Kakhuri are also cultivated in East Europe, Middle Asia, and other Caucasian countries.

Awareness of conservation for Georgian *V. vinifera* germplasm started since the nineteenth century (Staroselski 1893). During the twentieth century many grapevine collections were established in the country (Maghradze 2008). Between 2003 and 2010, significant progress was made in the frame of international research projects on conservation and sustainable use of grapevine (*V. vinifera* L.) genetic resources in the Caucasus and Northern Black Sea region aiming at strengthening the capacity of the region (Armenia, Azerbaijan, Georgia, Moldova, Russia, and Ukraine) to ensure long-term maintenance of *Vitis* genetic resources, including the cultivated traditional varieties and the wild gene pool (Bacilieri 2008; Bacilieri et al. 2010; Maghradze and Turok 2012). Beside conservation, the Georgian grapevine germplasm is involved in various scientific programs (Vouillamoz et al. 2006; Imazio et al. 2006; Maghradze et al. 2009a, 2010, 2012; Schaal et al. 2010; Myles et al. 2011).

Wild grapevine *V. vinifera* subsp. *silvestris* Beck., the wild ancestor of the cultivated grapevine *V. vinifera* subsp. *sativa* Beck., is a typical representative of the flora of Caucasus and Georgia. This plant is a component of almost all woody regions in Georgia, up to an elevation of 1,200 m (Ramishvili 1988). Nowadays, it grows in sparse small populations or even single individuals. All wild grapevine population suffered important genetic erosion, since the nineteenth century due to human activity expansion, pests, and diseases.

Few medieval references report existence of wild grapevine in this country (Sharden 1711). The first scientific investigation started in the middle of the nineteenth century as witnessed by the work of by Kolenati (1846). In the second half of the twentieth century (1956–1988), Ramishvili (1988) surveyed almost all regions and collected about 400 genotypes organizing them in ex situ field collections. The institute of Horticulture, Viticulture, and Oenology of Tbilisi was recently involved in an inventory, description, investigation and multiplication of wild vines project (Chkhartishvili et al. 2005; Maghradze et al. 2006a, b).

This paper reports the results of a study of genetic diversity and relationships both within the Georgian grapevine germplasm and a selection of representative grapevine varieties distributed worldwide using nuclear microsatellite markers. The advent of molecular markers offers a powerful tool to address these issues, as it was shown by previous works on grapes (Aradhya et al. 2003; Laucou et al. 2011) or other plant species (Harter et al. 2004; Vigouroux et al. 2005; Hamblin et al. 2007). Among these, SSR is the most prevalently utilized for genotyping individuals; solving problems of homonymy, synonymy, and kinship; and inferring the genetic structure of populations (Sefc et al. 2000; Grassi et al. 2003a; Cipriani et al. 2010).

To carry out precise and unbiased structure and parentage analysis, a set of 20 markers in linkage equilibrium was defined, selecting at least one locus per chromosome (Doligez et al. 2006). To be able to compare the local germplasm with a larger reference, we choose the same set that Laucou et al. (2011) used to characterize 2,323 different cultivars of the Vassal collection (http://www1.montpellier.inra.fr/vassal/, INRA France).

By combining historical and molecular approaches, checking whether local genetic groupings are concordant with the known history of the cultivars as well as their relations with the viticulture world, this paper aims to contribute to a better definition of the role played by Georgia in grapevine domestication and to contribute to an efficient preservation of old local genotypes that could represent valuable genetic combinations for a new and renewed viticulture.

Materials and methods

Plant materials

Cultivated accessions

One hundred twelve cultivated varieties were selected basing on previous works (Maghradze et al. 2009a, b) as representatives of Georgian native grapevine germplasm. All accessions are maintained in a grape germplasm repository in Gorizia province (Friuli-Venezia Giulia region, Italy) and 22 identified Georgian varieties are also available in the Institut National de la Recherche Agronomique (INRA) grape germplasm repository of Domaine de Vassal. The sample set is representative of 21 % of the total amount of traditional Georgian grape varieties (according to the already mentioned Georgian and Russian ampelography). Thus, it can't be considered exhaustive but most Georgian varieties, even the ones present in germplasm collections, lack complete ampelographic and molecular characterization and for this reason, only the true to type varieties were considered in this work.

Wild accessions

Eighteen true wild grapevines were also included in the analysis. All of them were selected in the spontaneous flora.

The main problem in sampling of wild *V. silvestris* grapevines is avoidance of false attribution to this subgroup of interspecific hybrids (*V. vinifera*×*Vitis* species) or feral *V. vinifera* accessions that escaped from cultivation. To avoid this kind of mistakes, the area of sampling should be far from vineyards (even abandoned ones) and plants should be checked for typical morphological characteristics such as leaf, berry, and seed dimension and shape, which differentiate the two subspecies (Anzani et al. 1990; Grassi et al. 2003b). According to the principles defined in the frame of the European GrapeGen06 project (http://www1.montpellier.inra.fr/grapegen06/accueil.php), the distances between wild accessions and cultivated vineyards were taken into account, and in the definition of each site of collection, a minimum distance of 10 km was considered. This measure was decided considering that the *V. vinifera* pollen grain of medium weight is not able to cover very long distances (Arnold 2002). To avoid the risk of collecting interspecific hybrids, differentiation between real wild vines and exotic ones from North America was carried out observing the main ampelographical descriptors and schemes on leaf and flower (Larrea 1978; Ocete et al. 2006); in addition, the SSR fingerprints were compared with the same data performed in other *Vitis* species. The 18 wild accessions were compared with a total of 53 samples belonging to different *Vitis* species conserved in the Vassal Germplasm collection and no match was found, neither identities nor kin. The most interesting and distinctive character is the flower structure: wild grapevines are dioecious while cultivated varieties are, in general, hermaphrodites. All wild samples were vegetatively propagated and are under cultivation in pots at the Milano University greenhouse facilities to allow continued morphological survey and characterization. This was particularly useful to define sex of wild samples (which is not always detectable in the wild) and allowed to calculate sex ratio. Sex attribution as well as other characteristics for wild and cultivated accessions is reported in Online resource 1a. In addition, few information regarding ecology of *silvestris* collection sites is given in Online resource 1b.

SSR genotyping

Samples of young leaves were collected for each accession during the active growing seasons in the field collection or from rooted woody cuttings in the greenhouse. DNA was extracted using Qiagen DNeasy Plant commercial kit. Twenty nuclear SSR loci were selected for their quality, polymorphism, and distribution across the 19 grapevine chromosomes based on the work of Doligez et al. (2006). These loci were detected on an automated ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Life Technologies, Foster City, CA, USA). Alleles were scored using GeneMapper 3.10 (Applied Biosystems, Life Technologies) and allele sizes were recorded in base pair with two decimal precision. After binning of alleles with Microsoft Excel, allele sizes were standardized to the sizes of reference varieties so the comparison could be made among collection datasets.

Data analysis

Diversity analyses were performed using the software GenAlEx (Peakall and Smouse 2006) to estimate, once redundant genotypes were excluded, the average number of observed alleles per locus (Na), the inbreeding coefficients (Fis), the observed heterozygosity (Ho), the average gene diversity and expected heterozygosity (He). The software IDENTITY 1.0 (Wagner and Sefc 1999) was used to estimate the frequency of null alleles (r) and the probability of identity (PI).

Genetic differentiation among groups of individuals was estimated by hierarchical analysis of molecular variance (AMOVA) basing on Fst values computed for our codominant data. F-statistics (Cockerham and Weir 1983) was performed with 100,000 permutations.

To describe the structure of these samples, a distance matrix based on Nei's GD was processed to obtain a neighbor-joining tree by PHYLIP package (Felsenstein 1989) and the dendrogram was displayed with TreeView 1.6.6. To visualize the genetic distances between accessions, PCA was carried out based on the matrix of genetic distance for codominant data (Smouse and Peakall 1999) and using GenAlEx software. Relationships between distance matrix elements were plotted based on their first two principal components.

Comparison between Georgian and Vassal collection grapevine samples This part of the work was devoted to the comprehension or the role played by Georgian viticulture in the definition of ampelographical platforms of different European countries. The Vassal grapevine repository was selected as representative of the worldwide grape cultivated varieties.

First of all, we were interested in evaluating if the 20 SSR loci were represented in the same way in samples from Georgia (*sativa* and *silvestris*) and other countries; for this reason, we compared the allele frequencies obtained in both cases and we performed a χ^2 test that we used to draw a tree linking together groups of accessions based on allele frequencies. Allele frequencies were also used to perform a PCA giving a spatial representation of distances among the four groups. Genetic distances among grapevines from Georgia and other countries were calculated using the Nei's index (1978) and a neighbor-joining tree was drawn in the same fashion previously described.

Finally, a parentage analysis was carried out, with the FaMoz software (Gerber et al. 2003) adapted to grape (Di Vecchi Staraz et al. 2007) to verify possible genetic relationships (parent–offspring (PO), half or full siblings) among

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the Georgian group of sampled varieties and the entire Vassal collection. A discrepancy of two loci was fixed to allow possible mistakes (Hoffman et al. 2005), the presence of null alleles (Dakin and Avise 2004; Wagner et al. 2006), or mutations (Riaz et al. 2002). The effect of scoring errors was also taken into account, searching relationships including possible mismatches (or incompatibilities) on a maximum of two loci, performed with FaMoz mistyping of 10^{-7} likelihood ratio, with a set of 20 markers.

The software method involves calculation of the logarithm of the likelihood ratio, log of odds ratio (LOD score), by determining the likelihood of an individual (or pair of individuals) being the parent (or parents) of a given offspring divided by the likelihood of these individuals being unrelated. LOD scores for any potential parentage relationship (parent/parent pair) with a value greater than zero are computed, giving statistical significance to the data.

Possible parents determined by logarithm of odds (LOD) scores and significance thresholds were probed among the 2,323 cultivars characterized with the set of 20 SSR markers (Laucou et al. 2011). Di Vecchi Staraz et al. (2007) determined, through 100,000 simulated parent pairs, a LOD score threshold of 8 for assessing a potential parent pair with 20 SSRs. Based on this, only pairs with LOD scores >8 were considered as valid in our work.

Uniparental most likely relationships are presented (relationship including only one parent and based on the fact that both individuals share at least one allele at each locus) with a likelihood ratio of the potential relationship. Most likely parent pairs are also displayed with the LOD scores.

Results

Genetic structure of Georgian germplasm (cultivated and wild accessions)

The 130 Georgian accessions were genotyped with 20 nuclear SSRs loci and inserted in the European Vitis Database. All the samples were collected in the same germplasm collection, but due to the existence of several common accessions (22) in the Vassal repository, a screening was made to verify the fingerprints. Each accession was represented by a single variety, with the exception of Badagi and Djineschi that were represented by two accessions both collected in Gorizia but coming from two different Georgian germplasm collections. In both cases, the two accessions were not coincident and we were able to select a putative true to type of Badagi comparing it with the Badagi accession present in the Vassal repository. In the case of Djineschi, this was not possible and both the accessions are marked not being true to types.

Allele frequency, observed heterozygosity, expected heterozygosity (He) or Nei's gene diversity, the fixation index (Fis) or inbreeding coefficient, null alleles frequencies (*r*), and probability of identity (PI) were calculated locus by locus and presented in Online resource 2. The average number of alleles per locus (Na) was higher in the cultivated dataset than in the wild one (14.450 vs. 8.750). However, the increase in allele numbers in the cultivated and wild samples did not significantly increase their effective allele number (Ne) that was quite similar in both cases. While the allele number largely depends on the complexity and size of the germplasm sampled, the effective number identifies those alleles occurring at a relevant frequency within the sample. In agreement with the results of allelic diversity, the expected heterozygosity or gene diversity (He) was also very similar in both groups, while the observed heterozygosity (Ho) was slightly lower for the cultivated samples than for the wild samples.

Figure 1 gives a graphical representation of the allelic patterns across the cultivated and *silvestris* groups. The inbreeding coefficient (Fis) was estimated at each locus; negative values were scored for eight out of the 20 SSR markers in the cultivated compartment and for 11 in the *silvestris* subset. The Fis values calculated for each locus indicate the absence of inbreeding or undetected null alleles affecting Georgian grape germplasm. The mean Fis values over loci for each compartment and for the total dataset were very close and not significantly different from 0.

Genetic differentiation among cultivated and wild compartments was estimated by hierarchical AMOVA. Results suggest that the largest part of differentiation has to be attributed to differences within the groups, both when considering the two subspecies (Fig. 2a) and also when splitting the cultivated compartment in eight different areas of traditional cultivation (Fig. 2b), while only 6 to 10 % of variation is due to variability among groups and among different geographical locations.

To verify the consistency of the gene flow from the Georgian *silvestris* compartment to the *sativa* vines, a population pairwise Fst estimate was computed (Table 1) and the resulting values were compared with the ones obtained in other recent works regarding grapevine domestication and gene flow between the two subspecies (Zinelabidine et al. 2010; Myles et al. 2011; De Andrés et al. 2012). On the other side, to visualize the genetic distances between accessions, PCA was computed based on the matrix of genetic distance for codominant data. The relationships between genotypes were plotted basing on their first two principal components (Fig. 3), accounting for 48.21 % of total variability, and different subsets were differentiated by both principal components. To verify the existence of a relationship among accessions and different regions of the country, Nei's genetic distances were computed among the nine

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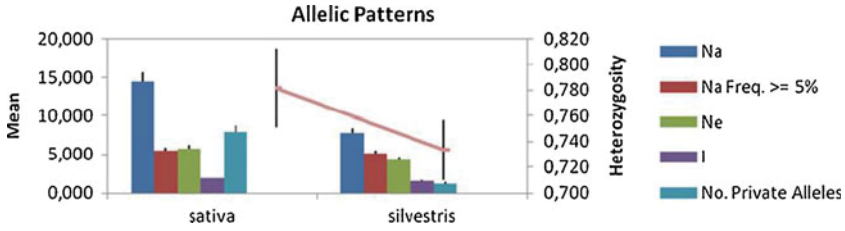

Fig. 1 Allelic patterns across the two groups. *Na* number of alleles detected, *Na*>5 % number of alleles with frequency higher than 0.5, *Ne* number of effective alleles, *I* Shannon information index

groups of accessions according to their major cultivation area. A neighbor-joining dendrogram, based on Nei's genetic distance, was built to give a graphical representation of the genetic distances among groups and to compare it to geographical ones (Fig. 4).

Parentage analysis and comparison with Vassal repository accessions

The allele frequencies obtained in Georgian *sativa* and *silvestris* groups were analyzed in comparison with the results discussed by Laucou et al. (2011), since this work examined 2,323 cultivars of the Vassal repository collection genotyped using the same 20 nuclear SSR. The χ^2 distance test was applied on allele frequencies to verify the distances among the groups. A dendrogram drawn based on the results of the χ^2 test among the four groups (Georgian: cultivated and wild; Vassal cultivated and wild) is proposed in Fig. 5. Based on allele frequencies, a PCA analysis was computed, and the coefficients for each allele at each SSR loci, describing the PCA representation shown in Fig. 6, are reported in the last three columns of Online resource 3.

To verify the link and possible relationships among Georgian cultivated grapevines and other foreign varieties, the Nei's genetic diversity was calculated in a dataset obtained combining SSR data for Georgian cultivated varieties and all the *sativa* samples inserted in the Vassal repository. A Ward (Ward 1963) dendogram was drawn and presented in Fig. 7 Finally, a likelihood-based approach was used to detect and explore most likely PO relationships among the Vassal repository samples and the Georgian accessions sampled for this work.

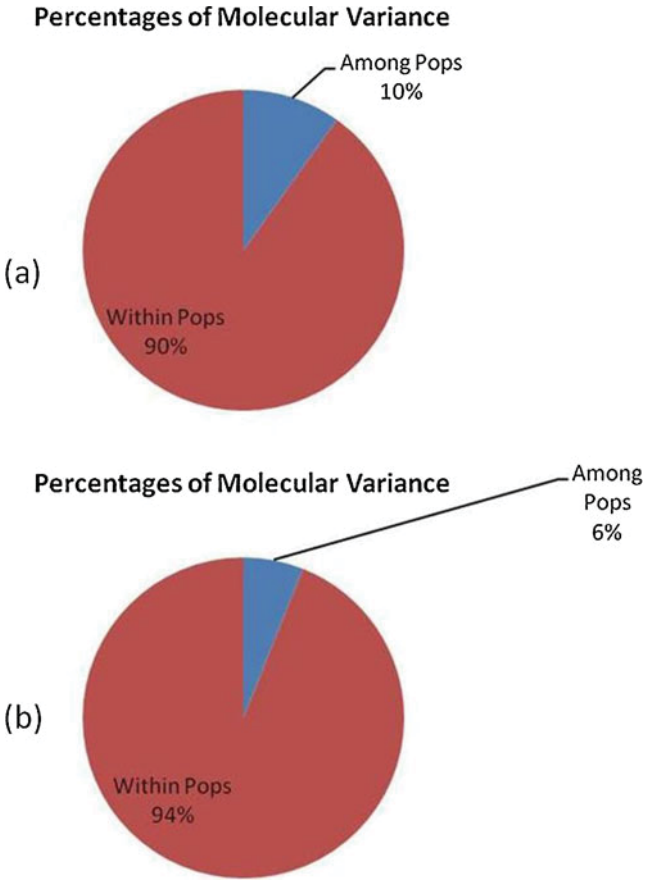

Fig. 2 **a**, **b** Analysis of molecular variance: defined with two populations (*silvestris* and *sativa*) (**a**) and among nine pops (*silvestris* and the cultivated accessions distributed in their eight proveniance regions) (**b**)

Discussion

Georgian germplasm structure and conservation

Number of alleles detected, observed, and expected heterozygosity for each loci were almost comparable with the ones evidenced in previous works devoted to the study of traditional grapevine cultivars from the South Caucasus and Anatolia (Vouillamoz et al. 2006; Maghradze et al. 2009a, b; Frare et al. 2011) and are in general agreement with the ones detected in cultivated and wild grapevine (Aradhya et al. 2003; Ibàñes et al. 2003; Sefc et al. 2003; Hvarleva et al. 2004; Zinelabidine et al. 2010; De Andrés et al. 2012). Number of alleles, varying from four to 27, respectively, at VVIn16 and VMC4f3 in our analyses, turned out to be comparable with the number of alleles and loci performed in the work of Laucou et al. (2011) on 2,323 cultivars. The mean value of expected heterozygosity (He) was of 0.786± 0.031 for the cultivated compartment and of 0.777±0.023 for the *silvestris* group. The cultivated value was comparable with the one detected in the work of Laucou et al. (2011) for cultivated samples (0.76±0.12), while the wild samples detected in the previously cited work performed a lower value (0.62±0.13). This result was quite unexpected, considering the higher number of *silvestris* (72 genotypes with

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Table 1 Pairwise population Fst values

	Abkhazeti	Adjara	*Silvestris*	Guria	Imereti	Kakheti	Kartli	Ratcha-Letchumi	Samegrelo
Abkhazeti	0.000								
Adjara	0.046	0.000							
Silvestris	0.076	0.069	0.000						
Guria	0.061	0.058	0.078	0.000					
Imereti	0.051	0.039	0.042	0.045	0.000				
Kakheti	0.061	0.046	0.056	0.074	0.028	0.000			
Kartli	0.069	0.047	0.055	0.080	0.031	0.017	0.000		
Ratcha-Letchumi	0.072	0.046	0.055	0.073	0.035	0.034	0.033	0.000	
Samegrelo	0.047	0.046	0.060	0.049	0.033	0.035	0042	0.047	0.000
	Sativa	*Silvestris*							
Sativa	0.000								
Silvestris	0.039	0.000							

single profiles among 80 analyzed) samples present in the Vassal repository compared with the 18 Georgian and also considering that wild accessions present in the French germplasm collection have different proveniences while the samples analyzed here all belong to the same local flora and places of sampling in the wild are not far the ones from the others. Moreover, the significant reduction of Ho, compared to He, usually experienced in Western European *silvestris* populations (Grassi et al. 2003b; Di Vecchi et al. 2006; Cunha et al. 2007; Lopes et al. 2009; Zecca et al. 2010; Zinelabidine et al. 2010; De Andrés et al. 2012) is not confirmed in the case of Georgian wild populations and individuals. These data are interesting because they indicate that Georgian cultivated and wild germplasm, despite isolation and low material exchange occurring during the past centuries, maintain a high level of gene diversity. This result

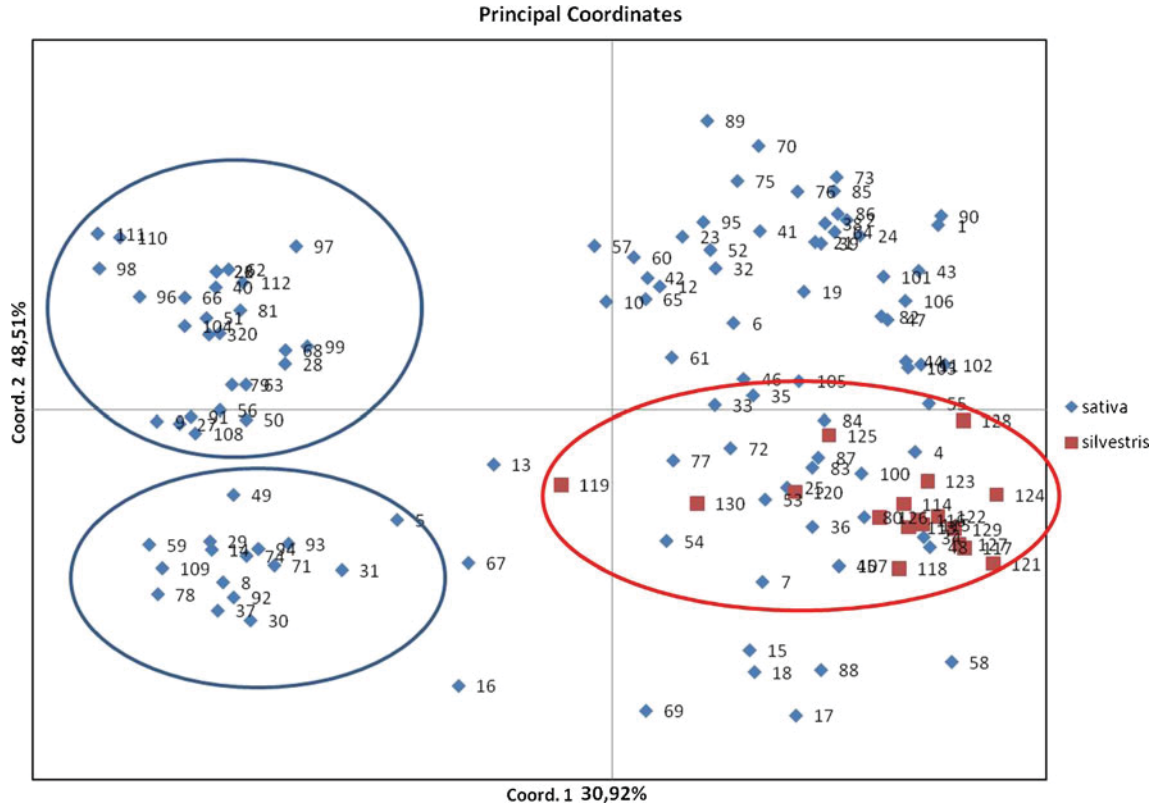

Fig. 3 Principal component analysis performed on Georgian samples (*silvestris* and *sativa*). *Red circle* evidences *silvestris* samples, while *blue circles* group samples that seem to cluster basing on their geographical proveniance

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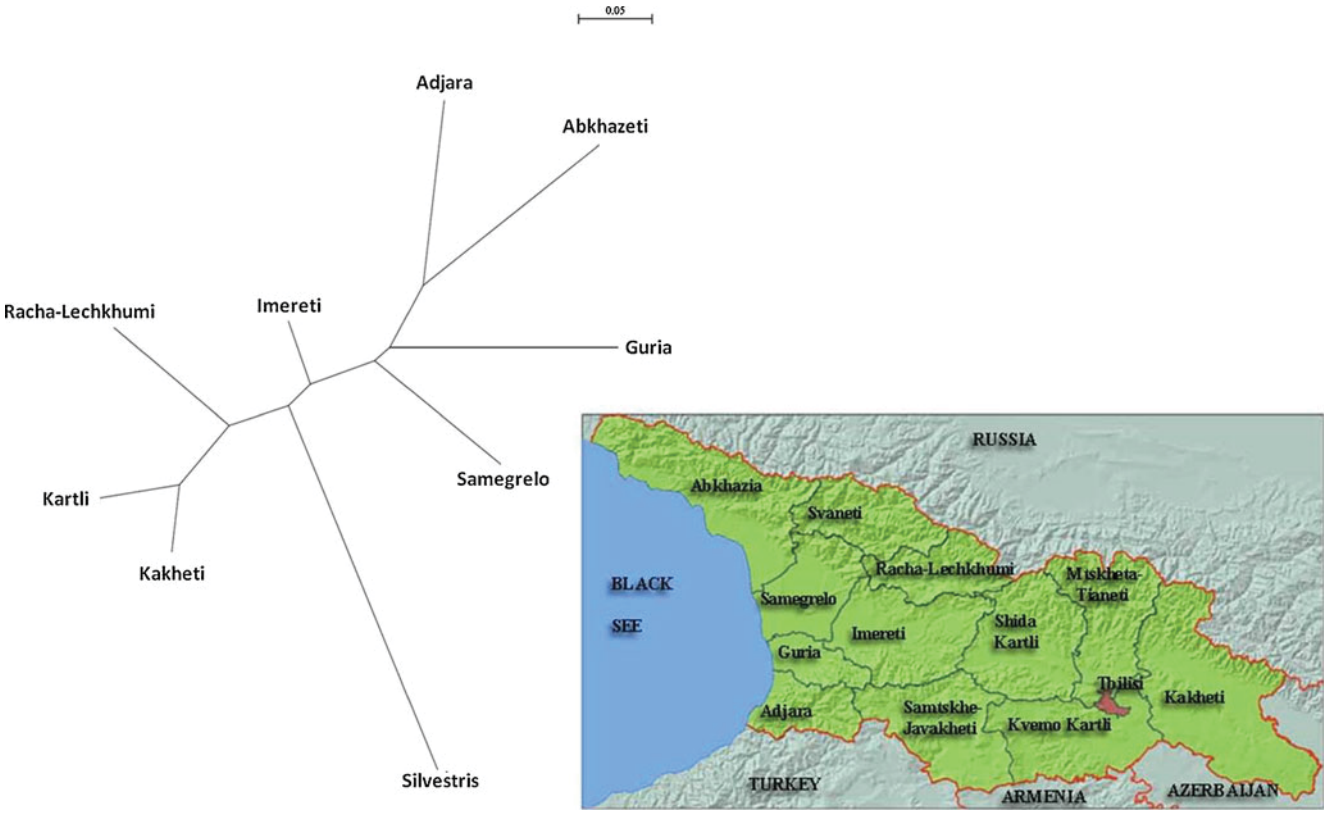

Fig. 4 Neighbor-joining tree computed on a Nei's genetic distance matrix and map of Georgia evidencing regions

is probably related to the long-standing cultivation tradition in Georgia (McGovern 2003a, b) but, first of all, it evidences the genetic richness contained in the wild *silvestris* compartment, underlining their good state of health.

The use of common loci was also useful in performing a locus by locus comparison of alleles detected in the datasets

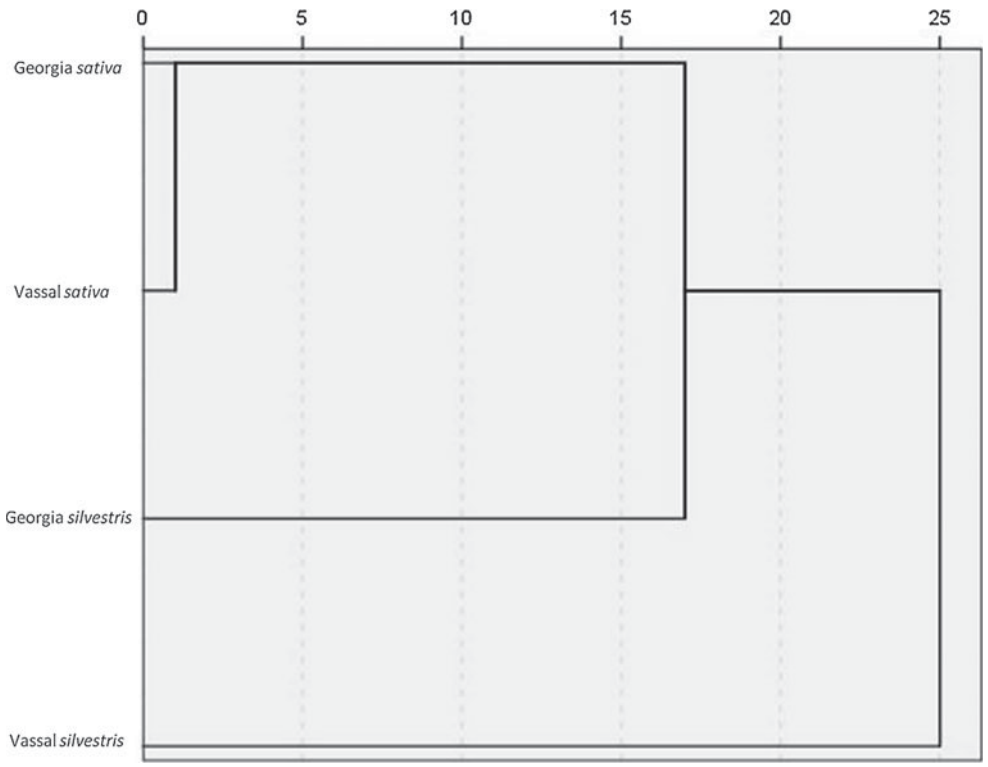

Fig. 5 Dendrogram drawn basing on the results of the χ^2 test among the four groups (Georgian: cultivated and wild; Vassal cultivated and wild)

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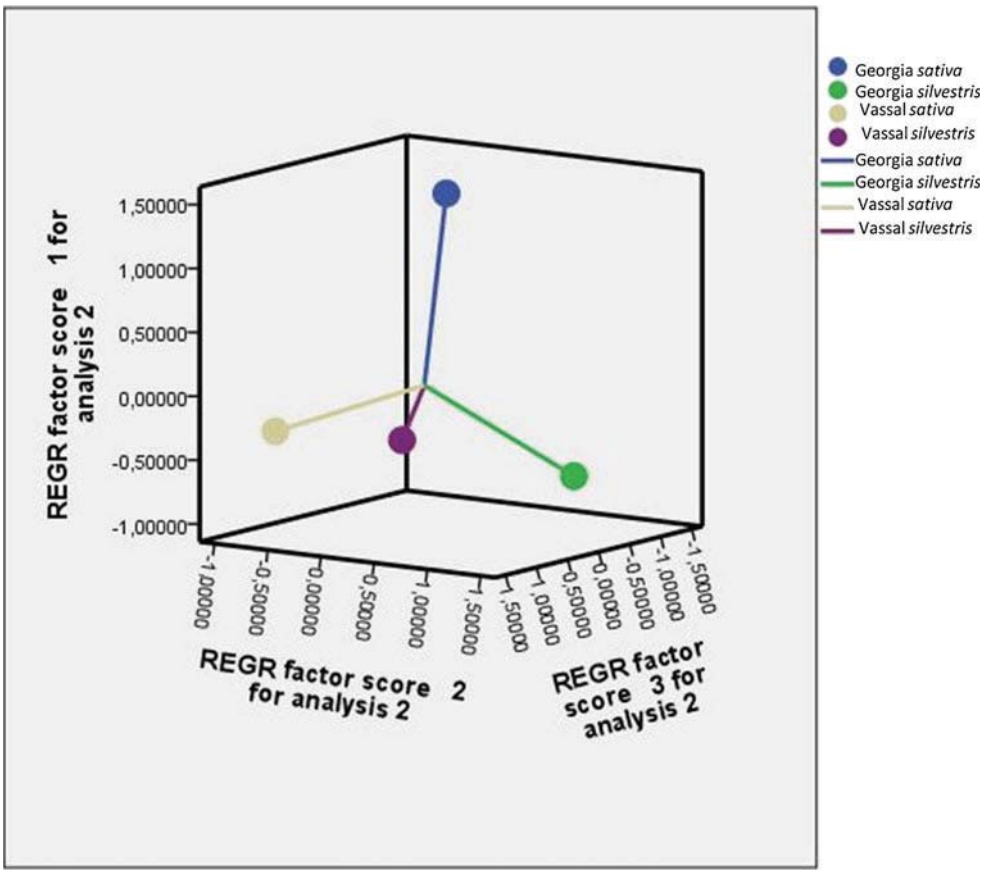

Fig. 6 PCA analysis was computed basing on allele frequencies. The eigenvalues for each allele at each SSR loci, describing the PCA, are reported in the last three columns of Electronic Supplemental Material 4

(Vassal and Georgia) and of their allele frequencies. The majority of allele frequencies (in all loci) from Georgian accessions were mainly coincident with the ones obtained in the Vassal repository (Online resource 3).

Among the alleles with different frequencies, overall loci, we were particularly mindful to those well represented in the Georgian dataset (*sativa* and *silvestris*) and absent or poorly represented in all the *V. vinifera silvestris* compartment described in Vassal, thus representing the wild *V. vinifera* populations collected worldwide. Among the 316 alleles identified in the 130 Georgian cultivars and in the 2,323 Vassal accessions, 73 alleles were presented in Georgian cultivars (with a frequency from 0.0044 to 0.1157) and not presented in Vassal accessions; just 15 alleles were present in the Vassal genotypes (with a frequency ranging from 0.0002 to 0.1048) and absent in the Georgian varieties. On the *silvestris* side, 59 alleles were present in the Georgian wild vines (frequency from 0.0217 to 0.5) and absent in Vassal wild vines, while the unique alleles were 48 (harboring a frequency range 0.0005–0.5347). These cases were present also in the most highly polymorphic SSR loci, thus reducing the risk of making erroneous suppositions based on a low number of alleles. The presence/absence of nuclear and chloroplast SSR alleles and the comparison of allele frequencies among countries have already been useful in the definition of the genetic structure of ampelographic European country platforms (Arroyo-García et al. 2006; Imazio et al. 2006). Studies on the Georgian viticulture seem to confirm, on one hand, the genetic richness of this viticulture and the originality of traits probably linked to local domestication events and on the other hand, the existence of the same alleles also in the cultivated compartment collected elsewhere and the contemporary absence in the wild dataset from the same countries could be considered a clue confirming the important role played by Georgian *V. vinifera* even in the formation of foreign varieties.

The detected allele frequencies for each of the four groups (wild and cultivated accessions from Georgia and Vassal) were used to perform a χ^2 distance test among the groups and to draw a dendrogram (Fig. 5), evidencing that the Georgian *silvestris* compartment is much closer to the cultivated varieties (Georgia and worldwide) than the Vassal *silvestris* is.

This last observation finds a confirmation in the recently published work of Myles et al. (2011), where a 9,000 high quality SNP array (*Vitis* 9k SNP array) was used to genotype 1,000 samples of *V. vinifera*. In this work, authors state that the haplotype diversity in western samples was slightly reduced compared with the eastern *vinifera*, and the population pairwise Fst estimates confirmed that all *vinifera* groups, considered in that work, were genetically closer to eastern *silvestris* than to the western one. Due to the absence of Georgian samples (no cultivated accessions

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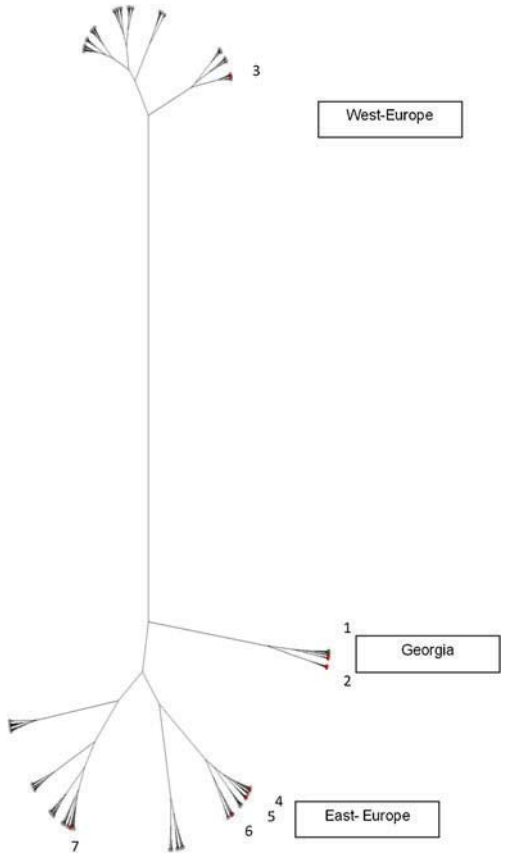

Fig. 7 Dendrogram based on the Ward method of hierarchical clustering, maximizing the between-group variance. It describes the distance dividing Georgian viticulture from other countries' grapevine platforms. In *green* (*1*), positions of the 23 Georgian wild grapevines, all grouping together. In *red*, the positions of the cultivated Georgian grapevines: (*2*) a group of 106 Georgian cultivated grapevines, grouping together; (*3*) a cultivar (*Tsulukidzis tetra*) close to Gouais or to Heunisch, and *4* to *7* Georgian cultivars grouping with cultivars from other East European regions [*4 Tsnoris tetra*, *5* Dzveli Aleksandrouli, *6* Djvari, *7* Saperavi, and Dzelshavi obchuri]

and only five wild samples were included) in that work, our results on Georgian wild and cultivated grapevines integrate the information given by Myles et al. (2011) and partially confirm their conclusions on the role of the Near East in grapevine domestication.

To verify in detail the role played by *silvestris* compartment in the constitution of the cultivated grapevine Georgian platform, we computed Fst population pairwise estimations (Table 1) both considering *vinifera* and *silvestris* as two separate groups and further splitting the *sativa* in eight different regional subgroups based on putative geographical provenience or the most prevalent growing area of each accession. Results evidenced lower values of Fst than the ones showed in previously published works (Zinelabidine et al. 2010; Myles et al. 2011; De Andrés et al. 2012) confirming a close relationship between *silvestris* and *sativa* individuals within Georgia. More details about this link were obtained by PCA presented in Fig. 3. Interestingly, the PC2 combined with low values of PC1 identified a differentiation in the *sativa* compartment and the individuals inserted in the two blocks are thought to belong to different geographical areas; the plants putatively originated in the eastern regions (Kartli, Kakheti) differ from the ones originating from the western part of the country (Abkhazeti, Samegrelo, Racha-Lechkhumi, Guria, and Adjara). This subdivision seems to reflect the geographical barrier constituted by Likhi Mountains connecting Major and Minor Caucasus, thus running in a north to south direction across Georgia and dividing the territory into two mayor parts. Remarkably, ampelographical characteristics, as leaf hairiness, seem to present differences among east and west varieties (Negrul 1946; Tsertsvadze 1989). The maintenance of phenotype and genotype differences, still able to distinguish the varieties based on their putative initial place of origin, is another confirmation that despite long-standing cultivation, traditional Georgian grapevines maintain their originality and local link to their main growing region.

On the other side, the first component of the PCA analysis defines two other groups (left and right hand sides of the graph). Interestingly, the group characterized by high levels of the second component comprises cultivated samples and *silvestris* accessions; thus, in the Georgian dataset, we are not able to clearly differentiate and separate *silvestris* and *sativa* compartments. Indeed, wild accessions are well distinguished from a part of the cultivated ones, but they are also completely overlapping with another subset of *sativa* accessions. The low Fst value (0.039) between Georgian *sativa* and *silvestris* groups finds here a graphical representation of the admixture existing between the two subgroups. Based on these considerations, two scenarios could be defined: the presence of "intermediate" cultivated genotypes may be explained by the presence of a gene flow between *V.v. sativa* and *V.v silvestris* (these genotypes being, thus, either first degree crossbreds or complex backcrosses) or, alternatively, by the hypothesis that the cultivated grapevine was derived through "domestication" by some genotypes and not others, thus implying that *V. v. silvestris* populations may not be homogeneous but rather contain at least two types of genotypes and just one was collected and analyzed in this work, the other one becoming extinct or simply not considered in this frame. We must report that Georgian ampelography is rich in cultivated varieties which names recall wild conditions, such as Tkis Vazi from Kakheti (East Georgia) and Tkis Kurdzeni from Adjara (West Georgia) where Tkis means forest, or bearing ampelographic traits (grapes and leaves) rather similar to wild vines, such as Tagidzura, Shavkurdzena, Opoura, Tchodi, Tsvrimala, Chitistvala Adjaruli, and Chrogha Kakhetis cultivars.

The positioning of *silvestris* genotypes in the PCA seems also to confirm, once again, the absence of interspecific

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hybrids among the wild samples collected. In fact, on the opposite, we should be able to identify *silvestris* samples isolated from the *V. vinifera* compartments, which is not. The neighbor-joining tree built upon Nei's genetic distance, among the nine data subsets confirmed the picture drawn by the Fst analysis. Moreover, results represented in Fig. 4 clearly show that genetic distances are directly proportional to regional distances: Kakheti and Kartli are two neighboring provinces in East Georgia, while Imereti, Samegrelo, Guria, Abkhazeti, and Adjara are neighboring regions of West Georgia. Georgia *silvestris* accessions included in this study were collected from East Georgia and, as expected, they have more similarities with the Eastern Georgia regions (Fig. 4).

All the considerations made above seem to underline the importance of the Georgian viticulture. First of all, the analyses accounted for high genetic diversity and variability. A quite unexpected result concerned the correlation of the genetic structure of Georgian germplasm with geographic locations, pointing to low germplasm and variety exchanges among regions and to the conservation of selected varieties in the regions. Our results seem to describe a scenario where grapevine cultivation has very old and strong cultural tradition and domestication seems to have occurred in different places in the frame of the country. All witnessed by the genetic richness still harbored in the cultivated varieties. To better investigate and comprehend the uniqueness of Georgian viticulture and to add information to the knowledge acquired on varietal circulation in the past, a comparison with cultivars from other countries was made, searching for contact points such as PO relationships and other clues, allowing detection of varietal exchange and admixture.

Parentage analysis and comparison with Vassal repository accessions

In spite of the distance, during all its history, Georgia kept relationships with European countries and peoples. Greek and Hellenistic colonies, documented on the Black Sea coasts of Georgia, played an important role in trades and cultural exchanges during the sixth to first centuries BC (Lomouri 1962; Lortkipanidze 1980); the Roman Empire was present in the Kartli region and in Colchis till the fourth century AD. The Byzantine Empire was a Georgia neighbor during the fourth to fifteenth centuries AD. Genoa Republic colonies on the Black Sea during the thirteenth to fifteenth centuries AD (Genoa 1979) and the Vatican catholic missioners in Georgia during the thirteenth to nineteenth centuries AD (Catholicism 1980) are documented as well. Furthermore, Georgia is strategically located: on the crossroad from Europe to Asia, between the Black and Caspian seas on the main trading roads, along the Great Silk Road (from the second century BC to the seventeenth century AD).

All these considerations increased curiosity in verifying the consistency of the putative role played by Georgian grapevines in the definition of the international grapevine platforms distributed worldwide.

First of all, the genetic distances with Vassal individuals were calculated (Nei 1978). This led to the tree represented in Fig. 7, where most of the Georgian accessions define a branch apart with apparently no admixture with other varieties belonging to other countries. Another interesting aspect of the scenario described in the dendrogram is that genetic distances seem to reflect geographical distances among countries, confirming once again that despite the knowledge about the existence of few primary domestication centers for grape, quite surely, local domestication events occurred at different times during grapevine history and evolution, and modern molecular genotyping techniques proved that they were of great importance in the definition of the actual genetic structure.

To better investigate these points, the FaMoz software, devoted to the reconstruction of pedigrees from molecular data such as codominantly inherited SSR markers, was used. The FaMoz software highlighted genetic relationships linking Georgian varieties. This data combined with ampelographic characterization and historical records should be used for cultivar classification. However, this ample target is out of the mission of the present article and the details will not be presented here. We also underline that the 20 SSR used in this work, even if they are uniformly distributed in the genome, are not enough to correctly attribute PO relationships, but the aim of this work was principally to elucidate the degree of admixture between viticulture of Georgia and other countries. Most of the relationships scored involved: Georgian material (data not shown) and Georgian accessions and varieties belonging to near or neighboring countries (such as Azerbaijan, Armenia, Russia, Iran, Ukraine, and Syria) and few interesting cases involving Georgian material and other European accessions as shown in Table 2. This result is not surprising, and these varieties could be particularly interesting in the frame of investigating grapevine domestication due to the fact that all the cited nations are considered putative areas of origin for grapevine cultivated varieties and could be interesting materials to address further investigations regarding genetic traits putatively selected through domestication such as berry pigmentation (Azuma et al. 2008; Lijavetzky et al. 2006) and aroma (Emanuelli et al. 2010). Other foreign countries involved in eventual PO relationships are France, Italy, Greece, and Austria. The most interesting aspect on this side is to define, when possible, the direction of these relationships; comprehending if the Georgian varieties involved are real autochthons of this country or if they have to be considered as introductions from abroad. Helpful, under this point of view, is the history of each variety combined with genetic information derived from the

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Table 2 Principal PO relationships identified between Georgian cultivated varieties and accessions present in the Vassal Repository and likelihood values (Only relationships with a LOD score>8 were considered)

Georgian variety	putative PO relationships (single parent)	LOD SCORE
ASURETULI SHAVI	RHODITIS (GREECE)	14.31
BUERA	GANZIANDY (ARMENIE)	33.75
DJVARI	MEHDIK (IRAN)	15.95
GOMIS TETRI	GANZIANDY (ARMENIE)	23.05
KAKHIS TETRA	PROSECCO TONDO (ITALY)	10.29
KHARISTVALA KOLKHURI	DURIF (FRANCE)	8.58
KHIKHVI	GANZIANDY (ARMENIE)	08.23
MAGHLARI SHAVI	ASSYLKARA (DAGESTAN - RUSSIA)	9.93
MARGULI SAPERE	ASSYLKARA (DAGESTAN - RUSSIA)	22.30
MARGULI SAPERE	BEKALNY (RUSSIA)	16.27
MSKHVILTVALA TETRI	GANZIANDY (ARMENIE)	8.87
PORTOKA	DURIF (FRANCE)	18.59
PORTOKA	SYRAH (FRANCE)	8.74
TSULUKIDZIS TETRA	PICCOLA NERA (ITALY)	15.83
TSULUKIDZIS TETRA	MEHLWEISS (AUSTRIA)	14.12
TSULUKIDZIS TETRA	GUEUCHE BLANC (AUSTRIA)	11.89
Georgian variety	putative PO relationships (parent couple)	LOD SCORE
ASURETULI SHAVI	RHODITIS (GREECE)XMAUVROUIDON (GREECE)	13.41
ASURETULI SHAVI	RHODITIS (GREECE)XKOTSIPHALI (GREECE)	11.66
BZVANURA	GANZIANDY (ARMENIE)XCHKHUTCHESHI (GEORGIA)	30.11
BZVANURA	DELI KAPTAR (TAJIKISTAN)XCHKHUTCHESHI (GEORGIA)	29.16
DJVARI	MEHDIK (IRAN)XMTSVANE KAKHURI (GEORGIA)	29.14
DJVARI	MEHDIK (IRAN)XRKATSITELI (GEORGIA)	23.95
DZIGANIDZIS SHAVI	MAGHLARI TVRINA (GEORGIA)XPETIT VERDOT (FRANCE)	16.68
GOMIS TETRI	GANZIANDY (ARMENIE)XBUERA (GEORGIA)	29.34
KAKIS TETRA	PROSECCO TONDO (ITALY)XKAISI BALADI (SYRIA)	12.35
PORTOKA	DURIF (FRANCE)XVIOGNIER (FRANCE)	17.85
PORTOKA	SYRAH (FRANCE)XDURIF (FRANCE)	10.13
TSULUKIDZIS TETRA	BLANK BLAU (AUSTRIA)XGUEUCHE BLANC (AUSTRIA)	23.01
TSULUKIDZIS TETRA	MEHLWEISS (AUSTRIA)XPICCOLA NERA (ITALY)	21.76
TSULUKIDZIS TETRA	GUEUCHE BLANC (AUSTRIA)XPICCOLA NERA (ITALY)	19.96

dendrogram described in Fig. 7, where some of these doubtful accessions are clearly not belonging to the Georgian branch. Particularly:

1. The variety Tsulukidzis Tetra, highlighted a strict relationship with the very well known Gouais Blanc. Gouais Blanc is a variety widespread in the Middle Ages in North Eastern France (Viala and Vermorel 1910), synonymous of Heunisch weiss variety, cultivated in Austria and, in the past, widely cultivated in Central Europe and especially in Dalmatia. Gouais Blanc has been proposed as a candidate for the grape given to the Gauls by Marcus Aurelius Probus (Roman Emperor 276–282), native of Pannonia. Another hypothesis claims the origin of Gouais Blanc specifically in Croatia (or Pannonia), but the Vitis International Variety Catalogue currently lists it as originating from Austria, which should probably be interpreted as "likely to originate somewhere in Central Europe." In the late 1990s, DNA fingerprinting at the University of California (Davis, CA, USA) identified Gouais Blanc as the ancestor of a large number of classical European grape varieties (Bowers et al. 1999). This sounds surprising given the old division into Frankish and Hunnic grape varieties used in the Germanic world, as it meant that the prototype simple Hunnic grape was, in fact, an ancestor to most of the noble Frankish grapes. Tsulukidzis Tetra was found to be sharing alleles in PO or sibling fashion also with other Austrian and Italian varieties, and according to the genetic distances defined in Fig. 6, it probably belongs to the western European viticulture, suggesting that the cited variety is not autochthonous from Georgia. The questionable origin of

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Tsulukidzis Tetra was already evidenced by some Georgian ampelographers (Cholokashvili 1938, 1939; Mirotadze and Bregvadze 1972; Ramishvili 1986) that considered this variety not autochthonous to Georgia and belonging to other European7 countries, despite other Georgian ampelographers convinced of the Georgian origin of this variety (Ketskhoveli et al. 1960; Tsertsvadze 1989; Ramishvili 2001). Particularly, according to Ramishvili (1986), it has to be considered as a synonym of the Spanish cultivar known as Albillo, also named Albillo Krismki, introduced in Georgia during the nineteenth century, but Tabidze (cit. in Mirotadze and Bregvadze 1972) supposed that it should be considered as derived from Pedro Ximenez Spanish cultivar and introduced in Georgia from Crimea. Our data are not able to support the synonymy supposed by the two authors but seem to confirm that Tsulukidzis Tetra is not a Georgian native variety. Moreover, the variety seems to have found a favorable environment in this country, and the probable first degree relationship with Gouais Blanc elevates its viticultural standing, becoming a half sibling of very famous international vines such as Chardonnay, increasing the interest and the need of a deeper investigation regarding this variety and the search of other putative Georgian candidates linked to Gouais Blanc offsprings.

In at least three other cases, our investigation led us to identify cases of a doubtful Georgian origin: Kharistvala Kolkhuri, Portoka, and Asuretuli Shavi.

2. Kharistvala Kolkhuri performs ampelographical traits (leaf shape, berry skin color, and plant habitus) not typical for the Georgian accessions, suggesting that a foreign origin could be involved and this might also explain the existence of a strict relationship with French varieties. The DNA fingerprinting confirmed ampelographic hypotheses about a foreign origin. The variety known as Portoka seems to have the same origin as Kharistvala Kolkhuri (same foreign varieties involved in the PO relationship); mostly, we have to highlight that no indication of the existence of this variety was found in the Georgian ampelography earlier than the one reported by Tsertsvadze (1988).
3. The case of the variety known as Asuretuli Shavi that has a relationship with the ancient Greek variety Rhoditis is also interesting. Asuretuli Shavi is a black-berried female variety from the Southern Georgia. According to Cholokashvili (1939) and Ortoidze et al. (2010), its origin is due to the discovery made by a German colonist within the period 1825–1845 in the woodland close to the village Asureti (Marneuli district, South Georgia) of a wildly growing, red-fruiting, and high-yielding plant. Nicolau and Michos (2009) described Rhoditis as a variety documented in Greece since the ninth century, hermaphrodite and with various biotypes. The contact of Georgian and Greek varieties is supported by the long history of communication between the two countries as previously reported.

For some of the Georgian varieties where foreign cultivars were involved, FaMoz software also evidenced putative parent couples, and for the varieties which historical information and ampelography do not support a Georgian origin (Portoka, Tsulukidzis Terta, Kharistvala Kolkhuri, and Asuretuli Shavi), the two individuals indicated as putative parents weren't Georgian, confirming that those varieties were probably imported in Georgia.

On the other side, the cases of relationships with neighboring countries (Table 2) regard principal varieties considered a part of the Russian, Iranian, Syrian, and Armenian viticulture very interesting. Of particular interest seems to be the role played by the variety Ganziandy (traditionally cultivated in Armenia) which, in some cases, combined with Georgian varieties, has a first degree relationship with some of the varieties analyzed in this work (Buera, Gomis Tetri, Mskhviltvala Tetri, and Bzvanura). Ganziandy is a wine variety, but little information is given in the Vitis International Variety Catalogue or in the European Vitis Database and even the recent Black Sea Ampelography (Del Zan et al. 2009) reports no description. Based on the USSR Ampelography (1966), the variety Gandziandy is a synonym of the Armenian wine variety Lalvari. The meaning of Gandziandy is not known, while the name Lalvari comes from a mount in the Armenian Lori Region. It is a rare variety, spread in the northeast of Armenia and not available in the South Ararat Valley (G. Melyan, personal communication). Lori is a border region of Georgia. This might explain why Ganziandy is present in the constitution of the Georgian ampelographical platform.

According to FaMoz, output the black berry wine variety Assylkara from Dagestan (Russia) seems to share a relationship with two Georgian varieties: Marguli Sapere and Maghlari Shavi. Dagestan, located in the north slopes of the Great Caucasian Mountains and neighbor to Georgia, is the most important viticultural region of Russia due to richness of grape germplasm. Asylkara is one of the oldest varieties of the North Caucasus (Stavropol' Krai, Dagestan), cultivated nowadays only in limited areas (18 ha) (Troshin and Radchevskii 2005). According to Marchenko and Peitel' (Ampelography 1946–1970), the variety Asylkara was probably introduced from the South Caucasus in the beginning of grape cultivation in the basin of river Terek.

Under this point of view, our indications, derived from SSR data and analyzed with the FaMoz software, correctly evidenced those varieties with a questionable origin, contributing to better knowledge on Georgian local germplasms and on those

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varieties needing a deeper investigation to verify their Georgian origin.

V. vinifera silvestris state of health in Georgia

The investigation carried allows addition of few considerations also on wild grapevine in Georgia, even if this is not the topic of the paper. The survey on Georgian wild grapevines has a long-standing history (beginning in the 60s of the past century) and is still in progress due to the activity of the Georgian Institute of Horticulture, Viticulture, and Oenology. All data obtained in these years were useful for the description of the main ecological traits regarding the typical habitat of wild grapevines in Georgia, which was very similar to the traditional environment found in all the other countries recording the presence of *V. vinifera silvestris*. As in other countries, it is quite difficult to find real populations: the common scenario is to identify sparse individuals. Among the 10 different places of collection, just for four of them were more than one individual recorded. In these four cases, the number of individuals for population varied from two to five. The sex ratio of all the samples investigated was near 1 with little prevalence of males on females, and if we consider the same ratio in each of the four populations identified (Online resources 1a), the proportion is respected with the only exception of Misaktieli population, where only females were accounted. However, we must underline that the limited number of individuals per population do not make these data robust enough to venture any conclusions on the fitness of each population and on their evolution. In addition, we must underline that till now only the western part of Georgia was interested in this kind of investigations. West Georgia is the most humid part of the country, thus harboring more vegetation than East Georgia, but in the next years, a deep survey also in the eastern part will be carried out in the frame of the European COST FA1003 project. This will be particularly useful not only to complete the description of the ecology of wild grapevine in Georgia but also to offer contribution to healthy aspects of this subspecies, especially regarding the state of health in relation to the major pests affecting *V. vinifera* species: Phylloxera (*Daktulosphaira vitifoliae* Fitch).

Despite belonging to the same species, Georgian *silvestris* individuals seem to be untouched by this pest. One of the most recent works published on this topic (Ocete Rubio et al. 2012) has verified the existence of disease symptoms in wild individuals only when the pest is directly and artificially inoculated. In the natural, wild environment, all the plants analyzed did not perform any symptom of the infestation. One of the reasons could be linked to edaphic conditions of the soils were the plants are hosted in the wild. Actually, *V. vinifera silvestris* lives in humid environments were the anoxic conditions of the soil are unsuitable for Phylloxera.

Conclusions

This work provides the first high-throughput analysis of Georgian viticulture and the first example of utilization of one of the more complete and exhaustive grapevine germplasm collections (Vassal Repository) as comparison base to verify and check out mistakes, synonyms, or false attributions in the frame of the viticulture of one of the most important countries for grape domestication. The work was carried out describing the genetic structure of cultivated and wild compartments present in Georgia and speculating about the existing links and admixtures with viticulture of other countries, trying to offer a contribution to the reconstruction of domestication events and routes that have designed modern viticulture in the way it is today. Our work offered strong confirmation to the most interesting and recent works addressing these issues. Particularly, the work of Myles et al. (2011) lead to conclusions about the role played by Caucasian countries that find here an important confirmation, and since the work cited had just few wild samples (# 5) and no cultivated varieties belonging to Georgia, our common conclusions based on other molecular markers (SSRs vs. SNPs) are particularly interesting, especially the ones regarding *silvestris* samples and their admixture with cultivated varieties.

All the results reported in the work are consistent with each other and all lead to the same description of Georgian viticulture as an example of an extremely conserved germplasm but, at the same time, it seems that no genetic erosions or drift have affected its structure. Evidences drawn in this work seem also to highlight a low degree of genetic exchanges with the rest of the world viticulture, even if not all the Georgian germplasm was inserted in our research. According to the Ampelography of Georgia (Ketskhoveli et al. 1960), Georgian germplasm is constituted by 525 varieties, among which most are characterized by the main ampelographical descriptors, so the number of accessions accounted in this work is just representative of the huge number of putative varieties. This last consideration does not invalidate our discussion and conclusions on Georgian viticulture. Oppositely, the high level of heterozygosis, the strict link with the terroir, and the existence of relationships with varieties from abroad not affecting the structure and characteristics of Georgian germplasm, achieved on a relatively low number of samples, are expected to increase when enlarging the sampling.

The avoidance of genetic assortment loss makes Georgian viticulture particularly interesting in the frame of genetic and agronomic studies. Vine cultivation and pest

diseases in the past had not affected the germplasm structure of Georgian cultivated varieties as in other countries.

This makes the country viticulture challenging when thinking at the possibilities offered by breeding for quality and/or resistance. Actually, in the last years, several Georgian native varieties were inserted in breeding programs in Georgia as in other foreign countries. As a result, 193 new varieties were bred in 15 countries, with the contribution of 13 Georgian native varieties (Vakhtangadze et al. 2010). Particularly interesting under this point of view seems to be the history of the Georgian cv Saperavi extensively used in Ukraine breeding programs (Goryslavets et al. 2010).

One of main constrains in the development of this kind of research is the few information (and for few varieties) achieved about agronomical characterization for Georgian varieties and the unavailability of this knowledge in European languages. This work aspires to highlight potentialities hidden in this unexplored germplasm and should be considered as a first step in the frame of increasing genetic assortments and variability for different purposes.

The search of information regarding varieties from Caucasian viticulture is also affected by the limited number of varieties from the region available in European germplasm collections. This limit, combined with the narrow varieties belonging to eastern European countries present in the same collections, affects also the search of possible relationships involving Georgian viticulture, making our results a possible underestimation of the real situation. The maintenance of Georgian and other Caucasian country viticulture is the first step, avoiding the loss, experimented in other countries, of local assortments. Proper knowledge of these genetic resources should prevent the loss of biodiversity. Also, the presence of new alleles found in the Georgian grapevine is a proof of its large diversity and should encourage more research for comprehension of genetic or metabolic features governing quality or resistance.

The need for improving the knowledge on grapevine genetic resources (cultivated and wild) is well underlined by the existence, in the last 10 years, of several research projects devoted to the exploration and characterization of biodiversity in this species. One of the last and most important is the GrapeGen06 European project (Bacilieri 2008; Bacilieri et al. 2010), where Georgia was included in addition to European countries, as a mark of the importance that the scientific community tributes to the grape germplasm of the country.

In crop species, only a small portion of the resources and of the genetic diversity conserved in germplasm is used by agricultural practice. To better investigate genetic resources hidden in the spontaneous flora and in the genomes of cultivated plants highly heterozygous as grapevine, field collections are the best strategy to verify single plant peculiar characteristics and to plan conservation and breeding strategies. This kind of maintenance is quite expensive and good management of genetic resources selected to be represented in the collection should be made. Only true to type material should be taken into account to optimize the number of genotypes. This is another issue that this work has started to consider for Georgian viticulture. The presence of accessions with the same name (or similar) in our group of samples and the existence of different fingerprints highlighting that they are not the same variety make urgent a collaborative work among germplasm repositories to rationalize the collections by developing proper common strategy also aimed to define and replicate core collections. This scenario and the results obtained and discussed in this work were the starting point for the definition of an EU-COST (http://www.cost.esf.org/domains_actions/fa/Actions/grapevine) project devoted to "East–west collaboration for grapevine diversity exploration and mobilization of adaptive traits for breeding." The new challenge raised in these last years is to explore the poorly known genetic resources still present in the presumed area of domestication for grapevine (southeastern Europe and particularly the Caucasus) and still enclosing untapped diversity and richness. The aim is to enable researchers from east and west European countries to introduce innovative areas of research at the European level, creating beneficial knowledge, long-term conservation, and greater quality of grape production in Europe.

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RESEARCH ARTICLE Open Access

Study of genetic variability in *Vitis vinifera* L. germplasm by high-throughput Vitis18kSNP array: the case of Georgian genetic resources

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Abstract

Background: Georgia, in the Caucasian region, is considered the first domestication centre of grapevine. This country is characterized by high morphological variability of cultivated (*Vitis vinifera* L. subsp. *sativa* (DC.) Hegi) and wild (*Vitis vinifera* L. subsp. *sylvestris* (Gmel.) Hegi) compartments. The main objective of this study was to investigate the level of genetic diversity obtained by the novel custom Vitis18kSNP array, in order to analyse 71 grapevine accessions representative of wild and cultivated Georgian germplasms.

Results: The number of loci successfully amplified was 15,317 out of 18,775 SNP and 79 % of loci resulted polymorphic. Sixty-eight unique profiles were identified, 42 for the *sativa* and 26 for the *sylvestris* compartment. Cluster analysis highlighted two main groups, one for cultivars and another for wild individuals, while a genetic structure according to accession taxonomic status and cultivar geographical origin was revealed by multivariate analysis, differentiating clearly the genotypes into 3 main groups, two groups including cultivars and one for wild individuals, even though a considerable overlapping area was observed.

Conclusions: Pattern of genetic diversity structure presented an additional proof that grapevine domestication events took place in the Caucasian region contributing to the crop evolution. Our results demonstrated a moderate differentiation between *sativa* and *sylvestris* compartments, even though a connection between several samples of both subspecies may be assumed for the occurrence of cross hybridization events among native wild populations and the cultivated accessions. Nevertheless, first degree relationships have not been discovered between wild and cultivated individuals.

Keywords: Domestication, Molecular markers, SNP, *V. vinifera* subsp. *sativa*, *V. vinifera* subsp. *sylvestris*

Background

Grapevine (*Vitis vinifera* L.) is one of the most widely cultivated species of agricultural interest [1], spread from Central Asia to the Mediterranean Basin [2]. Two subspecies, *V. vinifera* L. subsp. *sylvestris* (Gmel.) Hegi and *V. vinifera* L. subsp. *sativa* (DC.) Hegi, are considered to co-exist. The first one represented by wild populations and the second one represented by cultivated varieties obtained from wild individuals through a domestication process [3]. The two subspecies show differences in several phenotypic traits, one of the most distinctive traits is the flower sex, dioecious for wild grapes and hermaphroditic, or, to a lesser extent, female, for cultivated grapes [4].

The domestication of wild grapes started in the Neolithic Age, about 8,000 years ago, as a result of a long and gradual process closely linked to winemaking [5, 6]. Archaeological remains and proto-historical sources suggest the Near East area, comprising the South Caucasus, Oriental Anatolia, Syria and the area around Northern Mesopotamia, as the first centre of domestication [6, 7]. From the primary domestication areas, the grapevine spread to neighbouring regions and followed different pathways and successive waves firstly towards Mesopotamia, East Mediterranean Basin, North Africa, Southern Balkans and Aegean Region; secondly towards Sicily, Southern Italy, France and Spain; and

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finally towards Central Europe, mainly through the main trade routes of Rhine, Rhone and Danube rivers [6]. In agreement with these general dispersal pathways, many studies of grapevine genetic diversity supported the hypothesis of secondary domestication centres in the Mediterranean area, considering the crucial role of the Near East in grapevine domestication, and the introgression processes, from wild compartment of the secondary centres of domestication, in the cultivated germplasm, as complementary sources of genetic diversity in the domesticated gene pool [8-12].

A decisive contribution to interpret the molecular diversity of *V. vinifera* and its putative geographic origin was given by the analysis of two large grapevine collections [10, 13]. The first one repository, the grape germplasm collection of US Department of Agriculture (USDA, US) [10], includes over 1,000 *vinifera* accessions (table, wine and unknown type cultivars). The genetic variability of this collection, investigated by the Vitis9kSNP array (9,000 Single Nucleotide Polymorphism), showed a Near East origin of *V. vinifera* and presented evidence of introgression from local *sylvestris* individuals in the cultivated accessions along the European spread routes. The second collection analyzed was the largest grapevines repository located in Vassal (INRA, France) [13], counting for 2,323 unique genotypes representative of the grape growing areas around the world [14]. The microsatellite analysis revealed three main genetic groups and two additional groups, subdividing accessions according to geographic origin (Western regions, Balkans and East Europe, Caucasus and neighbour regions, Iberian Peninsula and Maghreb, Italy and Central Europe) and human use (wine and table grape cultivars).

Allowing the from-East-to-West trend, the genetic variability study of grapevine germplasm (130 grapevine samples representative of *sativa* and *sylvestris* compartments) coming from the first domestication centre, highlighted the uniqueness and originality of Georgian germplasm in respect to the worldwide accessions [12].

Since the '80s, different kinds of molecular markers increasingly more accurate, reproducible, repeatable, rapid and less expensive have been developed. The last frontier reached with the new generation sequencing (NGS) technologies is the high throughput SNP genotyping, a whole genome genotyping (WGG) assay that permits the economic and reliable screening of tens/hundreds of thousands markers per assay, leading the molecular characterization using SNP routine. SNP arrays were developed for apple/pear (*Malus pumila* Mill./*Pyrus communis* L.) [15], maize (*Zea mays* L.) [16], peach (*Prunus persica* L.) [17], potato (*Solanum tuberosum* L.) [18] and tomato (*Solanum lycopersicum* L.) [19]. Regarding grapevine, two different high throughput SNP arrays are available, the first one containing 8,898 SNPs [10] and the second one including 18,775 SNPs as part of the GrapeReSeq Consortium [20].

The main objective of this study was to investigate the level of genetic diversity, relationships and structure of dataset obtained by Vitis18kSNP array and to compare the usefulness of this new generation markers system in respect to the traditional SSR (microsatellite) used in [12]. We applied 18 k SNP descriptors, chosen in the frame of GrapeReSeq Consortium, to analyse 71 grapevine accessions representative of wild and cultivated Georgian germplasms, considered valuable genetic resources by the genetic and agronomic point of view.

Results

Genetic diversity

A total of 71 grapevine *sylvestris* and cultivated individuals representative of Georgian germplasm were analysed using the custom Vitis18kSNP array. Information about accession/cultivar name, region of origin, berry colour, flower sex, proles based on Negrul's observations [21], utilization and localization are given in Table 1 and Fig. 1.

The filtered dataset, after the removing of low quality and NC (non-call) loci, counted 15,317 out of 18,775 SNP loci successfully amplified. Among them, 12,083 loci resulted polymorphic, about 79 % of amplified markers. The final SNP allelic profile per each accession is reported in the Additional file 1: Table S1 and is available in Dryad repository [22]. Descriptive statistics for non-redundant genotypes were calculated and the distribution in *sativa* and *sylvestris* groups are summarized in Table 2. In the *sativa* group, were included also some accessions gathered as *sylvestris* but assign to the *sativa* compartment after cluster analysis (see below). The average number of effective alleles was 1.410 and the overall observed and expected heterozygosity values were respectively 0.293 and 0.289, while the percentage of loci showing minor allele frequency (MAF) values > 0.1 was about 73 % and the inbreeding coefficient (F) was 0.011.

The sex ratio (hermaphrodite:female:male) within the *sylvestris* compartment was evaluated (Table 3). The total sex ratio, among the seven populations, was higher for male individuals, followed by female and hermaphrodite (about 62:33:5). While, Sagarejo, Kvareli and Lagodekhi-Tbilisi populations showed the highest percentage of hermaphrodite, female and male flowers, respectively.

Cluster analysis

The genetic similarity among the different samples was calculated by Dice's coefficient (PEAS 1.0 software) [23, 24] and the grapevine accessions were grouped in clusters (MEGA 4.0 software) [25] as shown in Fig. 2. The genotypes showed different levels of similarity ranging from 86 and 100 %. Sixty-eight unique profiles were

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Table 1 List of cultivated and wild plant material from Georgia analysed in this work by 18 k SNP loci

ID	Samples	Berry colour[a]	Region of origin	Negrul's proles	Utilization[b]
	Vitis vinifera subsp. *sativa*				
1	Adjaruli Tetri	B	Adjara	*pontica*	W
2	Aladasturi	N	Guria, Imereti	*pontica*	W,T
3	Ananura	N	Kartli	*orientalis*	W
4	Argvetula	N	Imereti	*pontica*	W
5	Asuretuli Shavi	N	Kartli	*orientalis*	W, T
6	Bazaleturi	B	Imereti	*pontica*	W
7	Didshavi	N	Imereti	*orientalis*	W
8	Dziganidzis Shavi	N	Imereti	*pontica*	W
9	Gabekhouri Tsiteli	N	Imereti	*pontica*	W
10	Ghvinis Tsiteli	N	Kakheti	*pontica*	W
11	Gorula	B	Kartli	*orientalis*	T, W
12	Goruli Mtsvane	B	Kartli	*pontica*	W
13	Jani Bakhvis	N	Guria	*pontica*	W
14	Kamuri Shavi	N	Guria	*pontica*	T
15	Khushia Shavi	N	Imereti, Guria	*pontica*	W
16	Kvelouri	N	Imereti	*pontica*	W
17	Marguli Sapere	N	Imereti	*pontica*	W
18	Mgaloblishvili	N	Imereti	*pontica*	W
19	Mrgvali Vardisperi Kurdzeni	RS	Georgia	*orientalis*	T
20	Okhtoura	N	Kakheti	*pontica*	W
21	Orona	N	Guria	*pontica*	W
22	Paneshi	N	Samegrelo	*pontica*	W
23	Rkatsiteli	B	Kakheti	*pontica*	W
24	Rkatsiteli Vardisperi	RS	Kakheti	*pontica*	W
25	Rko Shavi	N	Imereti	*pontica*	W
26	Samarkhi	B	Guria	*pontica*	W
27	Sapena	B	Kakheti	*pontica*	W
28	Saperavi Atenis	N	Kartli	*pontica*	W
29	Saperavi Grdzelmtevana	N	Kakheti	*pontica*	W
30	Shavkapito	N	Kartli	*pontica*	W
31	Tamaris Vazi	N	Kartli	*orientalis*	W
32	Tavkveri	N	Kartli	*orientalis*	W
33	Tchumuta	N	Guria	*pontica*	W, T
34	Tchvitiluri	B	Samegrelo	*pontica*	W
35	Tita Kartlis	B	Kartli	*pontica*	T
36	Tkbili Kurdzeni	N	Kakheti	*pontica*	W
37	Tkvlapa Shavi	N	Imereti	*pontica*	W
38	Tkupkvirta	B	Kakheti	*orientalis*	W
39	Tskobila	N	Kakheti	*pontica*	W
40	Utskveti	B	Racha	*pontica*	W
41	Vertkvitchalis Tetri	B	Imereti	*pontica*	W
42	Zakatalis Tetri	B	Kakheti	*pontica*	W
43	Zerdagi (no true to type)	N	Samegrelo	*pontica*	W

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Table 1 List of cultivated and wild plant material from Georgia analysed in this work by 18 k SNP loci *(Continued)*

	Samples	Flower sex[c]	Region of origin (district, province)	Site category[d]	Distance from vineyards (km)
	Vitis vinifera subsp. *sylvestris*				
44	Bagitchala 05	M	Dusheti, Inner Kartli	A	10.0
45	Baisubani 01	M	Lagodekhi, Kakheti	C	3.0
46	Chachkhriala 01	fruits [F or H]	Akhmeta, Kakheti	AC	10.0
47	Delisi 04	M	Tbilisi, Inner Kartli	C	5.0
48	Delisi 06	M	Tbilisi, Inner Kartli	C	10.0
49	Kvetari 01	M	Akhmeta, Kakheti	C	10.0
50	Kvetari 05	F	Akhmeta, Kakheti	C	10.0
51	Kvetari 10	M	Akhmeta, Kakheti	C	10.0
52	Meneso 02	F	Dusheti, Inner Kartli	C	1.0
53	Misaktsieli 05	F	Dusheti, Inner Kartli	A	1.0
54	Nakhiduri 03	M	Marneuli, Lower Kartli	C	3.0
55	Nakhiduri 05	M	Marneuli, Lower Kartli	C	3.0
56	Nakhiduri 06	M	Marneuli, Lower Kartli	C	3.0
57	Nakhiduri 09	F	Marneuli, Lower Kartli	C	3.0
58	Ninotsminda 04	M	Sagarejo, Kakheti	C	2.0
59	Ninotsminda 08	H	Sagarejo, Kakheti	C	2.0
60	Ninotsminda 09	H	Sagarejo, Kakheti	C	2.0
61	Ninotsminda 11	M	Sagarejo, Kakheti	C	2.0
62	Ninotsminda 13	M	Sagarejo, Kakheti	C	2.0
63	Ramishvili 01	fruits [F or H]	Dighomi collection (Kartli)	-	-
64	Ramishvili 03	fruits [F or H]	Dighomi collection (Kartli)	-	-
65	Ramishvili 05	fruits [F or H]	Dighomi collection (Kartli)	-	-
66	Ramishvili 06	H	Dighomi collection (Kartli)	-	-
67	Ramishvili 07	F	Dighomi collection (Kartli)	-	-
68	Sabue 07	F	Kvareli, Kakheti	C	7.0
69	Sagubari 01	M	Akhmeta, Kakheti	A	6.0
70	Shirikhevi 04	fruits [F or H]	Dusheti, Inner Kartli	A	5.0
71	Zhinvali 01	M	Dusheti, Inner Kartli	AC	10.0

[a]B – Blanc (white), N – Noir (Black), RS –Rose (rose); [b]W - Wine grape; T - Table grape; [c]H - Hermaphrodite, F – Female, M – Male; [d]A - alluvial position (riverbank forest), C - colluvial position (slop of a hill), AC - both alluvial and colluvial positions

identified, 42 for the *sativa* compartment and 26 for the *sylvestris* compartment. Three pairs of matching genotypes were found, one among cultivars and two among sylvestris individuals.

Using the threshold value of 88 % for similarity index, two main groups were identified, one grouping cultivar samples and one for wild individuals. The 95 % of accessions were clusterized according to accession taxonomic status, except two cultivated genotypes (Tita kartlis and Utskveti, two of the most different genotypes) and two *sylvestris* individuals (Ramishvili 01 and Ramishvili 05) grouped in the *sativa* cluster. In the *sativa* cluster, the cultivars were arranged in two well distinct sub-clusters showing 87 % of similarity and including 18 and 24 unique profiles, respectively. The differentiation among cultivated and wild Georgian compartments was evaluated by Nei's genetic distance [26, 27] and Fst [28]. The two parameters reached 0.320 and 0.104 values, respectively.

Population structure analysis and differentiation

In order to identify the structure of populations and the correlations among samples, two different methods were performed. The first method was the PCoA analysis [29], computed based on the genetic distance matrix obtained by SNP profiles. Two dimensional projections of PCoA analysis per each sample were plotted in a 2-D dimension scattered plot (Fig. 3). The first two principal components (PCs), accounting for 25.63 and 18.29 % of the total variation, differentiated clearly the genotypes into 3 main groups, despite the presence of overlapping

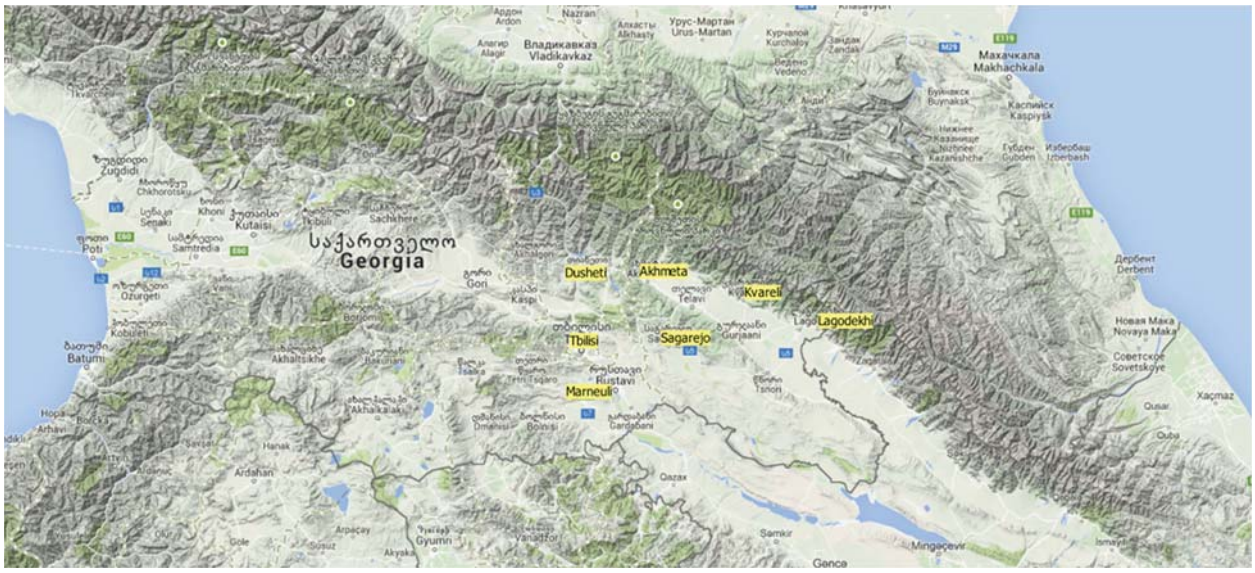

Fig. 1 Location of seven Georgian wild populations analysed. The tag of seven wild populations is yellow filled. The image is a Google Physical Layer created in QGIS 2.0

areas: two groups including cultivars (C1 and C2) and one for wild individuals (W1). In the overlapping areas, several cultivated samples appeared borderline with W1 samples. Along the PC1, a separation between C2 and W1 groups was highlighted, while the discrimination of C1 group was highlighted by the PC2.

The second method used to infer the relationship among genotypes was the clustering algorithm implemented in the fastSTRUCTURE program [30]. In order to uncover the hierarchical population structure, different numbers of K populations were explored (Fig. 4). Optimal K estimated the most likely number of populations at K = 3. Using a >0.75 % threshold for group assignation, 48 samples (68 %) were assigned to a cluster at K = 3 (Additional file 2: Table S2). Structure clustering highlighted 3 groups: two groups for *sativa* samples (G1 and G2) and one for *sylvestris* individuals (G3), including 25, 42 and 33 % of the entire genetic pool, respectively. In G3, only putative wild accessions (89 %) were included. The inbreeding coefficient (Fst) within three subpopulations identified by STRUCTURE analysis ranged from 0.076 (G1-G2 pairwise) to 0.064 (G2-G3).

Table 2 Genetic diversity of Georgian cultivated and wild grapevines revealed by 18 k SNP loci

Compartment/population	N[a]	Ne[b]	Ho[c]	He[d]	MAF[e]	F[g]
Sativa	47	1.396	0.312	0.297	24.433	−0.035
Sylvestris	21	1.519	0.278	0.329	13.932	0.161
Akhmeta	5	1.307	0.235	0.254	-	−0.087
Dusheti	5	1.326	0.246	0.270	-	−0.098
Kvareli	1	1.911	0.123	0.246	-	-
Lagodekhi	1	1.927	0.124	0.249	-	-
Marneuli	4	1.328	0.247	0.281	-	−0.141
Sagarejo	3	1.294	0.227	0.278	-	−0.203
Tbilisi	2	1.951	0.201	0.257	-	−0.289
Total	68	1.410	0.293	0.289	25.639	0.011

[a]Sample size; [b]Number of effective alleles; [c]Observed heterozygosity; [d]Expected heterozygosity; [e]Minor allele frequency: percentage of loci having MAF < 0.1; [g]Inbreeding coefficient; – not detected

Parentage analysis

Pairwise IBD (identical-by-descent) analysis was used to investigate the first-degree (PO: parent-offspring) and second-degree relationships among the wild and cultivated Georgian individuals by PLINK [31]. For an ideal situation without genotyping errors and/or mutations, Z0 (probability to share 0 IBD alleles) and Z2 (probability to share 2 IBD alleles) of PO pairs are expected to be 0 and Z1 (probability to share 1 IBD allele); Z0 and Z1 of 2nd degree pairs are expected to be 0.5 and Z2 to be 0. Therefore, pairs of genotypes holding a PI-HAT (relatedness measure) value similar to 0.5 are related by first-degree or closer relationships. Two pairs of individuals (Table 4) having Z0 and Z2 near 0, Z1 values higher than 0.9 and with relatively high proportion of IBD (PI-HAT ≈ 0.5) were considered PO pairs. One PO pair was identified between two wild samples (Ninotsminda 11 - Ninotsminda 13) and one between wild and cultivated samples (Ramishvili 07 - Tita kartlis). While, five pairs of samples (Table 4) with proportion of IBD (PI-HAT) ≈ 0.25 and relatively high Z0 and Z1 (≈0.5) values were considered 2nd degree pairs. The remaining pairs of individuals were considered "unrelated" according to

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Table 3 Percentage of male, female and hermaphrodite flowers in seven Georgian wild grapevine populations

Population (district)	Province	Number of individuals	Hermaphrodite (%)[a]	Female (%)	Male (%)
Akhmeta	Kakheti	5	0	40.00	60.00
Dusheti	Inner Kartli	5	0	60.00	40.00
Kvareli	Kakheti	1	0	100.00	0
Lagodekhi	Kakheti	1	0	0	100.00
Marneuli	Lower Kartli	4	0	25.00	75.00
Sagarejo	Kakheti	3	33.30	0	66.70
Tbilisi	Inner Kartli	2	0	0	100.00
Total		21	4.76	33.33	61.90

[a]Accessions classified with hermaphrodite or female flower were scored as female

the relationships identified. No 2nd degree relationships were identified among wild accessions and wild and cultivated samples.

Discussion

Genetic variability of Georgian *sativa* and *sylvestris* germplasms

In order to develop appropriate strategy for long-term conservation of the Georgian (and more general Caucasian) grapevine biodiversity, the identification and characterization of genetic resources is mandatory. There are not definitive data giving an estimation of the number of autochthonous varieties in this area: 525 varieties are listed in the Ampelography of Georgia [32], only 414 were described in the Ampelography of the Soviet Union (1947–1970), but only 248 remained in old collections until 2003 [33]. In the present study, the new Vitis18kSNP array, containing 18,775 SNP markers, were used to analyse the genetic relationship among a dataset of cultivated (43) and putative wild (28) grapevine accessions belonging to the autochthonous germplasm of Georgia.

The SNP statistic parameters calculated to determine the genetic diversity of Georgian germplasm reflected the results published in [12], regarding the genetic variability investigated by SSR markers. Considering the difference in the number of analysed accessions and the kind of molecular markers, the trend of Ne (number of effective alleles), Ho (observed heterozygosity) and He (expected heterozygosity) values between *sativa* and *sylvestris* compartments were almost comparable with the values evidenced in the previously cited work and in other works devoted to the study of cultivated and wild grapevines [11, 34]. For *sativa* compartment, the Ho value appeared slightly higher than the He value; while for wild accessions, the trend was opposite. The Ho reduction observed overall *sylvestris* samples and among populations was detected also by other studies [8, 34-39]. It indicated that the wild individuals suffer from inbreeding. This result was not observed for wild grapevine populations of Tunisia [40], as well as for the 18 spontaneous growing vines from Georgia analysed in [12]. The MAF value was higher for cultivated than wild samples, while, F showed mean value higher for *sylvestris* individuals (overall samples and among populations) than cultivars, and the same trend reported in [34] was displayed. MAF and F values were consistent with Ho results, showing that *sylvestris* compartment is more inbreed than the *sativa* compartment.

One of the main morphological distinctive traits between wild and cultivated grapevine forms is the flower sex, mostly hermaphrodite for cultivars and male or female for wild grapevine [4]. Moreover, hermaphrodite wild grapevine plants were also gathered. Subspecies *sativa* is self-pollinating, while subsp. *sylvestris* has an anemophilous and entomophilous pollination [41]. In nature, it was found a predominance of male wild grapevine individuals [42, 43]. Our results fit this evidence. Because of the flower of wild grapevines is unisexual and pollen of male plant fertilizes the ovary of female plant, the reproduction *via* sexual pathway of Kvareli, Lagodekhi and Tbilisi populations, where only female or male plants were collected, resulted damaged and these population are seriously endangered. Based on recent surveys in various European Countries [44-47], the wild grapevine populations appeared severely endangered and the reasons could be addressed to the human activities, ecosystem fragmentation events and spreading of Northern American pathogens. Nevertheless, in the natural environment, Georgian wild grapevine individuals did not show any signs of phylloxera attack. This could be explained because the existence of disease symptoms in wild individuals was verified only when the pest is directly and artificially inoculated [47].

Moreover, due to the limited number of individual per population our conclusions about their fitness are not really robust and have to be considered preliminary. Further surveys, devoted to explore in detail the spontaneous grapevine populations in Georgia and Caucasus as well, were conducted in the frame of EU project COST Action FA1003 “East–west Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding”. Fourteen wild populations

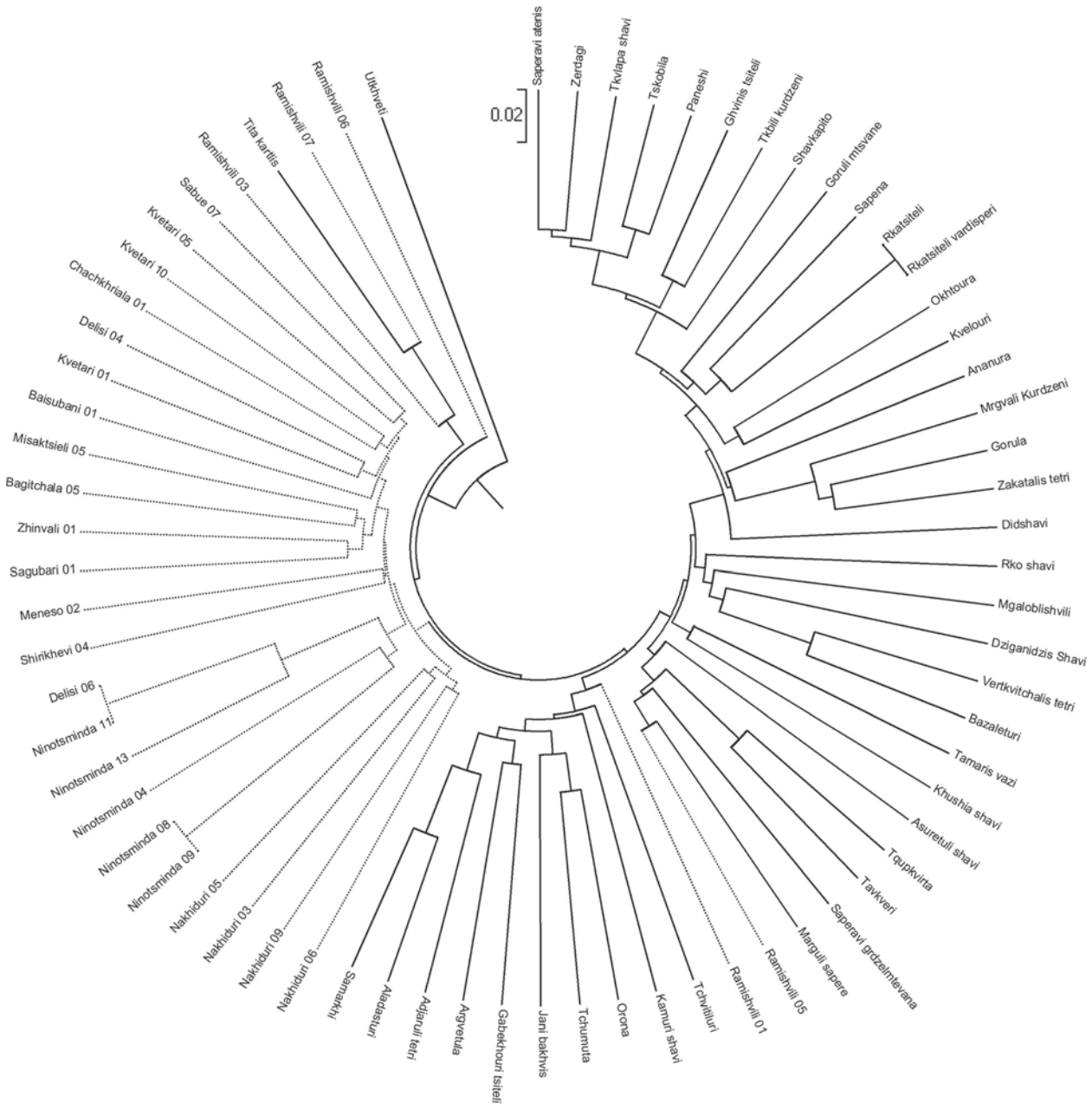

Fig. 2 Dendrogram showing relationships among cultivated and wild Georgian genotypes using 18 k SNP loci. Dendrogram generated using UPGMA method. Solid branch lines: cultivated Georgian genotypes; Dotted branch lines: wild Georgian genotypes

were investigated in their natural environmental (more than 100 individuals were sampled) and a prospecting on the sanitary status of the aerial organs and roots was carried out (Maghradze et al. accepted in Vitis). A genetic analysis including individuals coming from the latter surveys could give more exhaustive information regarding genetic diversity, fitness and inbreeding rates of grapevine wild populations in the Caucasus region.

In both *sativa* and *sylvestris* compartments, samples sharing the same allelic profile were found, for a total of 68 unique profiles identified (Fig. 2). Among the cultivars, the two samples sharing the same allelic profiles were Rkatsiteli and his berry colour mutant Rkatsiteli Vardisperi [12].

Rkatsiteli Vardisperi, a pink-wine grape, is a Rkatsiteli clone selected by V. Loladze in 1948 [48]. *V. vinifera* subsp. *sativa* is a cultigen vegetatively propagated through cuttings or budding. During this reproductive pathway, mutagenic events in the somatic cells of buds could take place and if they are used for propagation they lead to

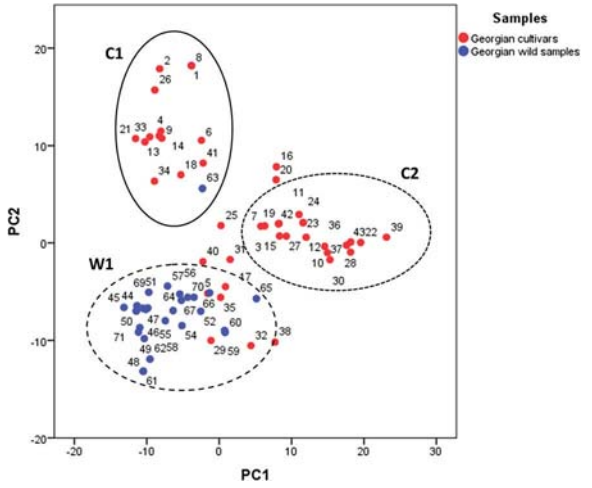

Fig. 3 Relationships between wild and cultivated Georgian samples as represented by the first two principal coordinates of PCoA using SNP profiles. C1: Western cultivars; C2: Southern cultivars; W1: wild individuals

genotype having phenotypic traits different to the mother grapevine. In the *sylvestris* compartment, two Ninotsminda individuals (08 and 09) collected in the same area, Sagarejo, shared the same allelic profile, while another accession (Ninotsminda 11) showed the same SNP profile of Delisi 06, an accession coming from Tbilisi, about 60 km far from Sagarejo (Fig. 2). The identification of two identical accessions (Ninotsminda individuals) collected in the same area could be addressed to a vegetative propagation event occurred to ensure a rapid vine regeneration and soil colonization. On the other hand, an error sampling could be highlighted for Ninotsminda 11 and Delisi 06.

In order to determine the genetic relatedness among genotypes, a clustering analysis was carried out (Fig. 2)

Table 4 Parentage analysis and relationship categories assignment (RCA) for wild and cultivated Georgian grapevines obtained by SNP allelic profiles

Sample 1	Sample 2	Z0[a]	Z1[b]	Z2[c]	PI-HAT[d]
RCA: Parent-Offspring					
Ninotsminda 11	*Ninotsminda 13*	0.0174	0.9015	0.0811	0.5318
Ramishvili 07	Tita Kartlis	0.0000	1.0000	0.0000	0.5000
RCA: 2nd degree					
Ghvinis Tsiteli	Tkvlapa Shavi	0.4841	0.4889	0.0397	0.2842
Mrgvali Kurdzeni	Zakatalis Tetri	0.4606	0.5076	0.0068	0.2606
Paneshi	Saperavi Atenis	0.5552	0.4709	0.0698	0.3053
Saperavi Atenis	Shavkapito	0.4807	0.5288	0.0163	0.2807
Saperavi Atenis	Tkbili Kurdzeni	0.4693	0.5103	0.0142	0.2694

[a]probability to share 0 IBD allele; [b]probability to share 1 IBD allele; [c]probability to share 2 IBD allele; [d]relatedness measure. *Italic type*: putative *V. vinifera* subsp. *sylvestris* individual

and the results were validated by pairwise Nei's genetic distance and Fst values. A clear differentiation regarding *sylvestris* and *sativa* compartments was recognized, using a threshold value for the similarity index lower than 87 %. Moreover, the result represented in Fig. 2 clearly showed that genetic distances are directly proportional to regional distances: the *sativa* samples were arranged based on the Western and Eastern origin, while the most part of *sylvestris* individuals were grouped according to their region of origin [12], *e.g.* Kvetari's, Nakhiduri's and Ninotsminda's.

The Utskveti variety, a cultivar clustering very distinct from the other ones, was interesting, as well as Tika kartkis variety, grouped together with Ramishvili wild individuals. The Utskveti variety was originated and widely spread in the past years in Racha province [49], but recently is only maintained in collections. The name of this variety was mentioned in the list of Georgian

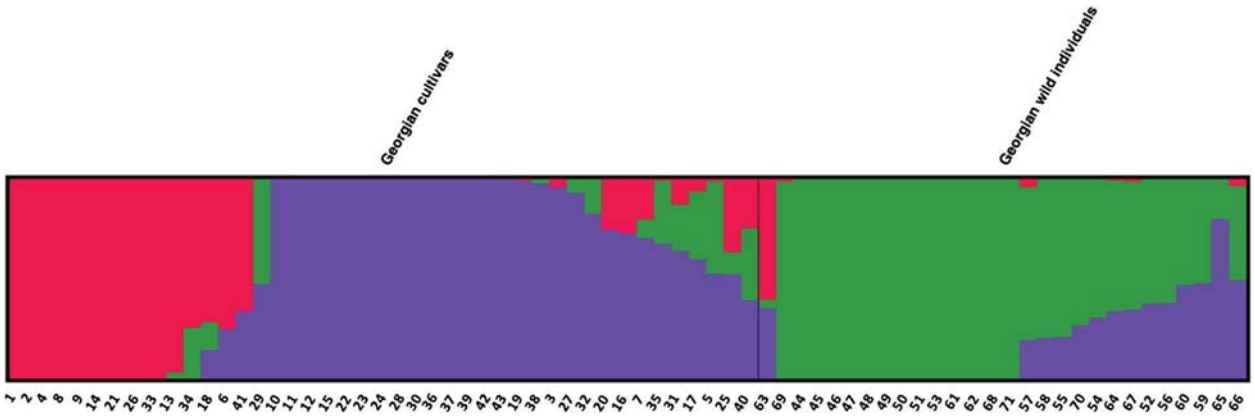

Fig. 4 Admixture proportions of wild and cultivated Georgian groups, as estimated by fastSTRUCTURE at K = 3, displayed in a barplot. Each sample is represented as a vertical bar, reflecting assignment probabilities to each of the three groups. G1: red bars; G2: purple bars; G3: green bars

local varieties [32] and the ampelographic description has been available since 1939 [50]. It is a white berry wine grape variety with strong hairs on lower leaf surface and with very dense bunches. The phenotypical observation of Utskveti accessions in the available Kindzmarauli, Telavi and Saguramo collections were only partially in agreement with the bibliography. Nowadays, the accessions have white berry and dense bunches but hairless lower leaf surface. Thus, some doubts about the correspondence of these accessions with historical Utskveti grape have to be accounted.

In the grapevine germplasm collections of Georgia are preserved two genotypes called Tita Kartlis. One is the true-to-type Georgian cultivar Tita Kartlis, having deeply lobed leaf and small prolonged berries [42] and the other genotype is the Azerbaijani cultivar Tabrizi, known in Georgia with synonym name of Ganjuri, differing from the Tita Kartlis true-to-type because of less lobed leaves, prolonged but larger berries and teeth in the petiole sinus [32]. Since the ampelographic description of the analysed accession in this study corresponds to the description reported in Ampelography of Georgia [32], the identification of Tita Kartlis is not questionable.

Taking into account that the Southern Caucasus (Armenia, Azerbaijan and Georgia) has been considered the first centre of grapevine domestication [7], the existence of local cultivars presenting morphological and genetic traits similar to wild individuals could be an instance of hybridization and introgression events among wild and domesticated accessions. Those events due to pollen flow between cultivars and wild forms were previously proved [11, 51] and could have severe consequences in the conservation of wild grapevine populations and advance the doubt if the current wild populations fit the ancestral grapevine forms [51]. Moreover, there are signs that only few Georgian cultivars could correspond to stocks introduced in the past from other neighbouring regions or far away countries, as France [12]. Despite the clear distinction between *sativa* and *sylvestris* compartments, few wild samples clusterized together with the cultivated samples. It is the case of Ramishvili samples, two grouped in the *sativa* cluster and three in the group of samples clusterized as outgroup. The Ramishvili samples have been collected by professor Revaz Ramishvili during his survey around Georgia in order to collect and study wildly growing grapevines. During this survey, not only wild grapes *V. vinifera* subsp. *sylvestris* were collected, but also accessions discovered in wild conditions during his expeditions and showing a phenotype holding typical ampelographic traits (grapes and leaves) of both *sylvestris* and *sativa* subspecies [52]. Based on cluster analysis, Ramishvili 01 and Ramishvili 05 could be considered cultivars because of their grouping in the dendrogram (Fig. 2). Regarding the accession Ramishvili 03, we do not have information about the flower sex, but we know it has white berries and we could conclude that it is not likely a *V. vinifera* subsp. *sylvestris* [53]. The accession Ramishvili 06 is hermaphrodite, whereby we could exclude its wild nature and classify it in the domestic compartment, as well as the accession called Ramishvili 07, having a female flower but not a wild habitus.

The identification of two well distinct clusters for Georgian samples were consistent with the high genetic variability and the genetic diversity of Caucasus germplasm coming from Georgia, considered a primary centre of grapevine domestication [7, 12, 13]. The high polymorphism of Georgian grapevines was also discovered by morphological characterization of *sylvestris* populations [54].

The two main groups obtained by cluster analysis were confirmed by Nei's genetic distance value (0.320), that it reflected the 87 % of similarity between the *sativa* and *sylvestris* clusters. This evidence was in agreement with the gene flow between the wild and cultivated compartments [11, 12]. On the other hand, the Fst value, accounting 0.104, meant that the two groups have a moderate differentiation based on the interpretation suggested by Wright [28]. This interpretation did not fit the low level of genetic differentiation between Georgian wild and cultivated grapevines revealed by using a moderate number of microsatellite loci [12, 55] or between Eastern *sativa* and *sylvestris* accessions analysed by 9 k SNP loci [10]. The latter discrepancy could be due to the absence of Georgian cultivars and the restricted number of Georgian wild individuals in the dataset.

Significant Fst values of genetic differentiation (about 0.140) have been reported between grapevine accessions of *sylvestris* and *sativa* in Morocco [38] and in Spain [11].

In agreement with the cluster analysis, the PCoA performed to identify the potential correlations among populations, revealed three main groups: C1, C2 and W1 (Fig. 3). Similar results, a clear distinction between *sativa* and *sylvestris* compartments, were also found analysing the Northern African germplasm by 20 nuclear microsatellites [40]. A differentiation of two separate clusters among Georgian cultivated samples was showed, confirming the existence of two genetic groups within the Georgian *sativa* germplasm, following the geographical provenience in the Georgian country described in [12] and [52], based on the molecular and morphological characterization, respectively. The samples collected in the Eastern regions of Georgia appeared separate from the accessions collected in the Southern and Western regions due to the orography and river basins functioned as biological boundaries. The overlapping area between C2 and W1 groups, slightly flattening the differentiation of cultivated and wild germplasm, was consistent with Nei's genetic distance value obtained between *sativa* and

sylvestris compartments and the discrete degree of similarity between the *sativa* and *sylvestris* subspecies [34], pointing out the existence of gene flow between both compartments [11, 12, 53]. Based on this evidence, it could be advanced the hypothesis of existing intermediate genotypes, having ampelographic characteristics inherited by both *sativa* and *sylvestris* subspecies, due to potential domestication events occurred in the past years in this area. Indeed, Ramishvili accessions could support this hypothesis: Ramishvili 05 was placed in between the C2 and W1 groups and Ramishvili 03, 06 and 07 accessions, considered *sativa* samples based on cluster analysis, in the PCoA plot belonged to W1. As well as, the clustering of six cultivars (Asuretuli Shavi, Marguli Sapere, Saperavi Grdzelmtevana, Tita Kartlis, Tavkveri and Tkupkvirta) in the W1 group led us to suppose that these cultivars were derived from local domestication events of *sylvestris* individuals. Contrary to what has been observed in this work, Asuretuli Shavi, a black berried female variety from the Southern Georgia (Marneuli district), was identified as a case of doubtful Georgian origin, because of based on SSR genotyping it showed a PO relationship with the ancient Greek variety Rhoditis [12]. Likewise the cluster analysis, Ramishvili 01 accession was grouped in one of the two *sativa* groups (C1). While Utskveti, the cultivars showing the highest genetic diversity in respect to the entire set of samples, was placed in the overlapping zone between C2 and W1. Furthermore, the distance between *sylvestris* sites and vineyards appeared to do not influence the overlapping area.

In addition to the major partition in cultivated and wild groups, STRUCTURE analysis identified three significant genetic groups, G1, including the majority of cultivars coming from Western region, G2, clustering *sativa* samples with predominance of cultivars coming from Eastern Georgia and G3, the group consistent with the wild accessions (Fig. 4). The STRUCTURE results, with 68 % of accessions clearly assigned to one group, recognized the genetic structure of Georgian germplasm (*sativa* and *sylvestris*), while the existence of samples showing an unclear assignation (less than 75 % of probability, Additional file 2: Table S2) could reflect the events of genetic introgression between wine-growing areas of Georgia. Considering the putative wild individuals analysed in this study, 14 out of 28 samples showed a percentage of assignation higher than 95 %, leading us to hypothesize that these wild individuals could be considered ancestral grapevine forms. Indeed, the accessions belonging to Ramishvili group were mostly included in G1 and G2 (Ramishvili 01, 05 and 06) and the other ones showed about 34 % of assignation to the Eastern Georgia group. The same six cultivars grouped into the W1 of PCoA plot were included in G2 and showed a not negligible percentage of assignation to G3. The pairwise Fst values higher than 0.05 among G1, G2 and G3 subpopulations revealed a moderate differentiation and the relatedness between Eastern and *sylvestris* individuals groups was confirmed by Fst lower value for G2-G3 pairwise. These results suggested that domestication events occurred in this geographic area as well as identified in [54, 55], where the STRUCTURE analysis, carried out on Georgian and wild accessions, revealed admixture among cultivated and wild samples, but a clustering regardless of their collection region was observed.

Archaeological evidence suggests that the grapevine domestication took place in South Caucasus and that its spread followed successive scenarios: the first one from Caucasus toward South-West (Eastern Mediterranean Countries), the second one toward Anatolia and after on the way to Greece, Balkans, Sicily, Southern Italy, France and Spain and the last one from France to Central Europe [7, 56]. Moreover, secondary centres of domestication have been proposed, as well as Iberian Peninsula, where it was found the chlorotypes of *sylvestris* and *sativa* genotypes compatible with Western cultivars chlorotypes [9], and Italy, where the allelic profile of some cultivars was found very similar to some wild accessions [57].

Even though a connection between some *sylvestris* and *sativa* individuals was highlighted by both multivariate and STRUCTURE analysis, the kingship analysis did not find out close relationship between wild and cultivated samples, because of Ramishvili 07, showing a PO relationship with Tita Kartlis, is now considered a *sativa* individual. Nevertheless, if introgression events occurred between the two subspecies and parental individuals were not analysed, the parentage relationships higher than 2nd degree are difficult to identify. Moreover, it cannot be excluded that close relationship could be discovered between two subspecies enlarging the number of analysed accessions. The 1st degree relationship between two wild samples (Ninotsminda 11 and Ninotsminda 13), located in sites not far from each other is consistent with propagation events by seed dispersal [58] and confirmed the inbreeding tendency in some wild populations.

In a time characterized by great challenges to face climatic change and to develop sustainable agricultural models based on use of moderate irrigation, fertilisation and pesticides, the selection of new genotypes for ensuring an optimal productivity in terms of quality and quantity is mandatory. It was demonstrated that the Georgian grapes are late ripening cultivars, characterized by a long vegetative and reproductive development (from bud break to harvesting time) in comparison with Western European cultivars [59]. The objective to select varieties showing a wider range of phenological variability and genetic traits, apparently not represented in the

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germplasm of Western Europe, makes the Georgian var-ieties a considerable background for grapevine breeding programs aimed to extend the ripening time in a viticul-tural area and consequently reducing possible berry sum-mer stresses and grapes quality impairment.

Considering the grapevine defence against diseases, a survey about use of fungicides in member states of the European Union highlighted that viticulture accounts for approximately 70 % of all agrochemicals used. Nevertheless, an intensive use of chemicals becomes more and more unsustainable because of high costs, and possible negative impact on environment and human health due to the chemical residues in grapes, soil and aquifers. The EU Directive 2009/128 for sustainable control of diseases caused by plant pathogens in Europe strongly recommends a decrease in the number of pesticide treatments carried out in the field. Thus, following the first interesting results obtained by screening the Caucasian germplasm [60], a systematic investigation of Georgian grapevine genetic resources, searching for resistant traits to pathogen, seems to be a promising strategy for plant breeding programs aimed to reduce the fungicides use in vineyard assuring at the same time an acceptable protection against pathogens.

SNP and SSR molecular markers in comparison

Vitis18kSNP array is the largest SNPs set implemented in a high-throughput genotyping technology for genetic diversity in grapevine. The previously SNPs sets included tens [61], hundreds [34] or thousands loci [10]. SNP platforms have been developed following the huge genomic data obtained by sequencing and re-sequencing of whole genomes using NGS technology on accelerated pace, which allow high-throughput and low cost genotyping of thousands of markers in parallel.

On the other side, SSR markers are a useful instrument widely used for genotyping, to solve problems of homonymies, synonymies and kinships, to infer genetic structure of populations in wild and cultivated grapevines [11-14, 32, 62, 63]. A set of 9 SSR markers was proposed as minimal set of loci for genotyping routine analysis [14] and for parentage analysis or for germplasms not covered by this set an additional group of 13 SSR loci was included [14, 63].

In this work, it was demonstrated that the SNP markers were useful for germplasm management, as already observed in grapevine [10] and in many other species [15, 17, 18] and that the results could be compared to other marker systems, as the traditional SSR [12, 55]. Moreover, SNP markers revealed a higher differentiation, pointing out a moderate differentiation between *sativa* and *sylvestris* compartments based on Fst value, and at the population level the high number of loci should solve better the genetic relationship among samples. In respect to SSR markers, these microarray-based markers were used to investigate helpfully the genetic diversity of Georgian *sativa* and *sylvestris* germplasms with a limited expense in terms of time and money and obtaining a high data reliability (only the 18 % of loci showed low quality or were not detected). Moreover, since the SNPs are biallelic the genetic profiles could be easily compared to datasets generated by other laboratories around the world, without incurring problems related to difficulty on data standardization [64].

Another winning aspect could be the application of Vitis18kSNP array for parentage analysis. Nowadays, the parentage analysis works are carried out including dozens of SSR loci [63] and, sometimes, even by increasing the number of analysed loci not all the relationships discovered previously can be ruled in [65]. An in-deeper analysis, using thousands of SNP loci, could strengthen the data obtained by kinship analysis, mostly for second and third-degree relatives, for which more than 50 SSR loci should be investigated for the detection [66].

Furthermore, this array could successfully be chosen for the construction of high-density maps, quantitative trait loci (QTL) mapping, genetic diversity and parentage analysis in grapevine.

Conclusions

The results obtained by molecular analysis of Georgian germplasm using a large set of SNP markers provided information of high genetic diversity of *sativa* and *sylvestris* Georgian germplasms, as previously investigated by other molecular markers and by morphological evaluations. Our data showed that the Vitis18kSNP assay can be used successfully for high-throughput SNP genotyping in grapevine and represented a viable alternative to traditional genotyping techniques. According to this work, a moderate differentiation between *sativa* and *sylvestris* compartments was discovered, due to centuries long separation of two taxa, making it quite impossible to trace the events of *V. vinifera* domestication. On the other hand, connection between samples of both subspecies may be assumed as well, highlighting the occurrence of cross hybridization events among native wild populations and cultivars.

Methods

Plant materials and DNA extraction

In this study, 43 cultivated samples and 28 putative wild accessions coming from Georgia and maintained in the germplasm collections of University of Milano were considered. A detailed list of plant material is reported in Table 1. About *sylvestris* accession sampling, refer to Material and Methods described in [12]. Seven grapevine wild populations were taken into account in this work, distinguished on the basis of some parameters, such as

sharing of the same area, distance between groups (more than one linear kilometer) or the presence of geographical barriers (Fig. 1). Ramishvili samples were covered as *sylvestris* individuals from Dighomi collection (Kartli, Georgia). Accessions were classified in the *V. vinifera* subsp. *sylvestris* taxon according to their expected morphological traits, mainly related to the young and mature shoots and leaves, flower type and bunch aspect at flowering and during ripening, berry and seed size and shape. This morphological analysis allowed also to discriminate among true *V. vinifera* and possible non *V. vinifera* species or inter-specific hybrids. In particular accessions were considered genuine wild *V. vinifera* if they showed: i) fully opened young shoot apex; ii) low anthocyanin coloration and density of hairs, both on young shoot apexes and leaflets; iii) mature leaves small or medium in size, with short teeth, low density of hairs and open petiole sinus; iv) small bunches; v) small and round berries; vi) roundish pips.

Total genomic DNA was extracted by young leaves using the DNeasy™ Plant Mini Kit (Qiagen - Hilden, Germany). In order to determine the DNA quality, the 260/230 and 260/280 ratios was detected by NanoDrop Spectrophotometer (Thermo Scientific - Waltham, Massachusetts). Quant-iT PicoGreen Assay (Invitrogen - Carlsbad, California) was used to quantified the DNA concentration.

SNP genotyping

The 18,775 SNPs contained in the Vitis18kSNP array (Illumina Inc., San Diego, California) were analysed. Two hundred nanograms of genomic DNA were delivered to Fondazione Edmund Much (San Michele all'Adige, Trento, Italy) and were used as template for the reaction, following the manufacturer's instructions (Illumina Inc.). Nucleotides were scored with Genotyping Module 1.9.4 of the GenomeStudio Data Analysis V2011.1 software (Illumina Inc.). Dataset was filtered based on SNP call quality and GenTrain score: samples with low SNP call quality ($p50GC < 0.54$) were removed from the analysis and only SNPs with a GenTrain score higher than 0.6 were retained. Markers with a number of NCs (no-call) higher than 20 %, as well as the 100 % NC markers, were removed. The data can be downloaded from Dryad repository (De Lorenzis et al. [22], http://dx.doi.org/10.5061/dryad.521h5).

Data analysis

In order to estimate the genetic diversity of Georgian germplasm, the SNP genotyping data were used to determine the effective number of alleles (Ne), the observed heterozygosity (Ho), expected heterozygosity (He) [67], the minor allele frequency (MAF) and inbreeding coefficient (F), performed by PEAS 1.0 software [23]. The sex ratio of *sylvestris* individuals was calculated among and within populations, estimating the percentage of hermaphrodite, female and male flowers.

MEGA software (version 4.0) [25] was used to design a phylogenetic tree by the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method. The SNP distance matrix was generated by PEAS 1.0 software [23] based on the Dice's coefficient [24]. The validation of clustering results was performed considering the pairwise Nei's genetic distance [26, 27] and pairwise Fst analysis [28]. The parameters were carried out using the pp.fst function of HierFstat package [68] and nei.dist function [69] of R program.

The structure and the association between *sativa* and *sylvestris* Georgian compartments were investigated following two different approaches: i) Principal Coordinates Analysis (PCoA) [29], used to capture the correlation between genotypes; ii) STRUCTURE analysis [30], a Bayesian approach attempts to interpret the correlation between genotypes in terms of admixture between a defined number of ancestral populations. The PCoA analysis was carried out by GenAlEx 6.501 software [69], starting the correlation matrix. The STRUCTURE analysis was carried out using fastSTRUCTURE software package [30], using the input files (.bed, .bim, .fam) generated by PLINK 1.07 software [31]. K (number of ancestral genetic groups) values, ranging from 1 to 10, were tested by 10 iterations per each K and the most likely K value was chosen, running the algorithm for multiple choices of K. The admixture proportions estimated the most likely K was viewing by DISTRUCT software [70]. The K clusters obtained by STRCUTURE analysis were validated performing pairwise Fst values [28].

In order to infer relationships among individuals, we employed the PLINK 1.07 software [31] on each pair of all the genotypes (only unique genotypes were included), estimating the proportion of the SNPs at which there were 0, 1, and 2 shared alleles identical-by-descent (IBD: probability of two genotypes are descended from a single ancestral genotype and not identical by chance), denoted by Z0, Z1, and Z2 respectively and PI-HAT values, the relatedness measure measured as PI-HAT = P (IBD = 2) + 0.5 x P (IBD = 1). The parameters, minor allele frequency (MAF) and r^2 of linkage disequilibrium, were set on 0.01 and 0.05 values.

Availability of supporting data

The data set supporting the results of this article is available in the Dryad repository, (De Lorenzis et al. [22], http://dx.doi.org/10.5061/dryad.521h5) and as complementary material (Additional file:1: Table S1).

Additional files

Additional file 1: Table S1. SNP allelic profile of 43 grapevine cultivars and 28 putative wild individuals from Georgia at 15,317 SNP loci. Dataset resulted was filtered based on SNP call.

Additional file 2: Table S2. Ancestry values (mean and standard deviation values over 10 interations) for the three genetic groups inferred by structure on 43 grapevine cultivars and 28 putative wild individuals from Georgia genotyped at 15,317 SNP loci.

Abbreviations

A: Alluvial position (riverbank forest); AC: Both alluvial and colluvial positions; B: Blanc (white); C: Colluvial position (slop of a hill); F: Female; F: Inbreeding coefficient; Fst: Fixation index; H: Hermaphrodite; He: Expected Heterozygosity; Ho: Observed Heterozygosity; IBD: Identical-by-Descent; M: Male; MAF: Minor Allele Frequency; N: Noir (Black); N: Sample size; NC: Non-Call; Ne: Number of effective alleles; NGS: New Generation Sequencing; PC: Principal Coordinate; PCoA: Principal Coordinate Analysis; PI-HAT: Relatedness measure; PO: Parent-Offspring; QTL: Quantitative Trait Locus; RS: Rose (rose); RCA: Relationship Categories Assignment; SNP: Single Nucleotide Polymorphism; SSR: Simple Sequence Repeat; T: Table grape; W: Wine grape; WGG: Whole Genome Genotyping; Z0: Probability to share 0 IBD allele; Z1: Probability to share 1 IBD allele; Z2: Probability to share 2 IBD alleles.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

GDL participated in the design of the study, performed DNA extraction, SNP genotyping, data analysis and wrote part of the manuscript. RC collected wild material. OF conceived the study, participated in the design of the study and wrote part of the manuscript. DM participated in the design of the study, collected wild material and wrote part of the manuscript. All authors read and approved the final manuscript.

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RESEARCH ARTICLE Open Access

Genetic diversity analysis of cultivated and wild grapevine (*Vitis vinifera* L.) accessions around the Mediterranean basin and Central Asia

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Abstract

Background: The mountainous region between the Caucasus and China is considered to be the center of domestication for grapevine. Despite the importance of Central Asia in the history of grape growing, information about the extent and distribution of grape genetic variation in this region is limited in comparison to wild and cultivated grapevines from around the Mediterranean basin. The principal goal of this work was to survey the genetic diversity and relationships among wild and cultivated grape germplasm from the Caucasus, Central Asia, and the Mediterranean basin collectively to understand gene flow, possible domestication events and adaptive introgression.

Results: A total of 1378 wild and cultivated grapevines collected around the Mediterranean basin and from Central Asia were tested with a set of 20 nuclear SSR markers. Genetic data were analyzed (Cluster analysis, Principal Coordinate Analysis and STRUCTURE) to identify groups, and the results were validated by Nei's genetic distance, pairwise F_{ST} analysis and assignment tests. All of these analyses identified three genetic groups: G1, wild accessions from Croatia, France, Italy and Spain; G2, wild accessions from Armenia, Azerbaijan and Georgia; and G3, cultivars from Spain, France, Italy, Georgia, Iran, Pakistan and Turkmenistan, which included a small group of wild accessions from Georgia and Croatia. Wild accessions from Georgia clustered with cultivated grape from the same area (*proles pontica*), but also with Western Europe (*proles occidentalis*), supporting Georgia as the ancient center of grapevine domestication. In addition, cluster analysis indicated that Western European wild grapes grouped with cultivated grapes from the same area, suggesting that the cultivated *proles occidentalis* contributed more to the early development of wine grapes than the wild vines from Eastern Europe.

Conclusions: The analysis of genetic relationships among the tested genotypes provided evidence of genetic relationships between wild and cultivated accessions in the Mediterranean basin and Central Asia. The genetic structure indicated a considerable amount of gene flow, which limited the differentiation between the two subspecies. The results also indicated that grapes with mixed ancestry occur in the regions where wild grapevines were domesticated.

Keywords: Domestication, Genetic structure, Microsatellite, *V. vinifera* subsp. *sativa*, *V. vinifera* subsp. *sylvestris*

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Background

Vitis vinifera L., the commonly cultivated grapevine, is one of the most widely grown fruit plants in the world [1]. It has subspecies with West Asiatic and European origins, and ranges from Central Asia to the Mediterranean Basin [2]. Within the genus *Vitis*, *V. vinifera* is the primary species used in the global wine industry, which occupied 7.5 million hectares in 2012 and produced more than 67 million tons of grapes (http://www.oiv.int/). Within this species, two subspecies have been described, *V. vinifera* subsp. *sylvestris*, which includes the wild populations, and *V. vinifera* subsp. *sativa*, which includes the cultivated varieties that resulted from the domestication of the wild relatives [3]. The main phenotypic traits that distinguish the subspecies are: flower sex (dioecious for wild populations and hermaphroditic, or rarely female, for cultivated grapevines); and the seed morphology (spherical seeds with a small beak for *sylvestris* and pyriform seeds with a well-developed beak for the domesticated cultivars) [4, 5]. The two subspecies form a genetic and taxonomic continuum without breeding barriers resulting in spontaneous hybrids where they occur sympatrically or paripatrically [6–12].

Pioneering work of Negrul [13] divided the grapevine cultivars into three groups or *proles*: *occidentalis*, *pontica* and *orientalis* depending on geographic distribution and morphological and ecological differences. Grapevines found in the wide area extending from eastern Georgia, Armenia, Azerbaijan, and the former Soviet republics in Central Asia to the Near East have clear distinguishing features and were placed in the *proles orientalis*. Negrul recognized two sub-*proles* within this main group: *caspica*, composed of ancient vines used for vinification before the advent of Islam (from CE 500–1100), and the *antasiatica* including table and raisin grape cultivars of more recent origin. Varietal ecotypes found from Georgia to the Balkans were designated *P. pontica* sub-*proles georgica* and sub-*proles balkanica*, respectively.

Grape domestication occurred about 8000 years ago, during the Neolithic Age and was closely related to advances in winemaking in the Near East and area around Northern Mesopotamia [14–16]. The dissemination of grapevines from the primary domestication center into neighboring regions of Europe and Northern Africa followed three main pathways, first toward Mesopotamia, reaching the Southern Balkans and East Mediterranean Basin (end of the fifth millennium BCE), then toward Sicily to Western Europe and, finally, domesticated grapes were introduced to Central Europe during the first millennium BCE [16]. Meanwhile, during the fourth century BCE grapevine cultivation reached Central Asia, and near the second century BCE domesticated grapes were introduced into China and Japan [14, 15].

The cultivated grape *V. vinifera* subsp. *sativa* has played an important economic and cultural role throughout human history in different parts of the world. However, its ancestor the European wild grape *V. vinifera* subsp. *sylvestris*, is close to extinction. To capture and maintain the existing genetic diversity, researchers from East and West European countries under the framework of COST Action FA1003 (East-West collaboration for grapevine diversity exploration and mobilization of adaptive traits for breeding) initiated efforts to collect and preserve germplasm from a wide range of countries, including regions where autochthonous germplasm had not been investigated by genetic and ampelographic methods [17, 18].

The wild relatives of crop species have great importance to breeders as unique sources of genetic variation for breeding programs [19]. Wild grapevines are normally found in riparian ravines where they have access to water and can climb into the tree canopies. One impact of increased human population pressure is the destruction of natural habitats of wild flora and rapid erosion of genetic diversity. There is urgent need to characterize and conserve this valuable germplasm for future generations, and to design a strategy to preserve this species ex situ through extensive collections of wild grape that capture the genetic variation present in the Mediterranean basin and Central Asian regions. A closer analysis of Central Asian collections revealed that many genotypes resist fungal disease, such as downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*), and black rot (*Guignardia bidwellii*); all of which were supposedly introduced from North America about 150 years ago [20]. Other studies found that plants of *V. vinifera* subsp. *sylvestris* located in an area of Spain with heavy metal contamination exhibited high tolerance to copper stress [21]. Biotic and abiotic stresses from new pathogens, pests and a changing climate have spurred the creation of better-adapted varieties. Adequate genetic variation is the key to breeding crops capable of resisting these challenges.

Molecular analysis has provided insights into the genetic diversity of *V. vinifera* in relation to wild relatives, the genealogy of cultivars and the specific alleles linked to selected traits [15, 22, 23]. Although Central Asia is one of the centers of grapevine diversity, the majority of information about this region's germplasm has emerged from accessions maintained in European and USA germplasm repositories [10, 12, 24]. The genotyping of wild and cultivated accessions from a broad range of viticultural areas at two large grapevine repositories provided a significant dataset capable of elucidating relationships within and between the two subspecies at the global level [10, 25]. Results from these studies suggest that grapevine spread from East-to-West after the first domestication process. The results also provide evidence of introgression from local *sylvestris* individuals with cultivated accessions [25], and the impact on genetic structure related to geographic origin and human use [10].

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A limitation of previous examinations of grape genetic diversity was unbalanced sampling resulting in a germplasm collection set that was limited to one or more countries and was not broadly representative. In addition, the *sylvestris* and wild germplasm from the Caucasus Mountains and Central Asia was poorly represented or not analyzed in these studies. Although genetic, archeological and linguistic evidence suggests that southern Anatolia was the cradle of grape domestication, Transcaucasian remains a serious candidate as evidenced by ancient grape remains that were excavated from Neolithic archaeological sites in Azerbaijan as well as in Georgia [5]. Therefore, the results of previous studies may not present a complete picture of relationships between the wild and cultivated grapevine groups in that region and their association with the rest of world. The first large-scale characterization of both wild and domesticated grapevines, was done by Imazio et al. [12], utilizing SSR (Simple Sequence Repeats) fingerprint data from a set of 382 wild and 130 cultivated grapevine samples collected from Georgia. The results found four genetic groups, two for wild accessions and two for cultivated genotypes. The accessions from Georgia were included in a separate clade that highlighted the uniqueness of Georgian germplasm. Two other studies of grape germplasm from the Caucasus region also found that both wild and cultivated grapes had high genetic and morphological diversity [26, 27].

A previous study by Bacilieri et al. [10] analyzed genetic diversity of 2096 cultivated genotypes maintained in the Vassal germplasm collection and suggested the original center of grapevine domestication extended into many Central Asian countries. A comprehensive study that includes samples from the wild and cultivated groups, collected from opposing sides of an East-West gradient, and samples from Central Asian countries would provide a better understanding of the impact of geography and human selection on grapevine domestication and adaptive introgression. It would further allow us to determine the overall relationships of germplasm within the centers of domestication and with their wild progenitors. With these objectives, data were pooled from six previous studies {Laucou et al. [7], De Andrès et al. [8], Imazio et al. [12], Riaz et al. [24], Biagini et al. [28], Zdunić et al. [29]} and new data were generated for wild accessions collected from Croatia, Georgia, Armenia and Azerbaijan, to develop a well-balanced set that represented both subspecies and provided maximum representation of key geographical regions [Mediterranean basin and Central Asia (Spain, France, Italy, Croatia, Georgia, Armenia, Azerbaijan, Iran, Turkmenistan and Pakistan)]. SSR data were analyzed to infer the genetic structure of populations in wild and cultivated grapevines and to determine the role of Central Asian grapevine germplasm in the diversification of the cultivated gene pool. Results are discussed with emphasis on the conservation of wild germplasm tolerant to biotic and abiotic stress and its use in breeding programs.

Methods

Plant materials

A total of 1378 wild (*V. vinifera* spp. *sylvestris*) and cultivated (*V. vinifera* spp. *sativa*) samples from Transcaucasia (Armenia, Azerbaijan and Georgia), the Caspian Sea region (Turkmenistan and Pakistan), and Europe (Croatia, France, Italy and Spain) were included in the study. Table 1 and Additional file 1: Table S1 present a detailed list of the analyzed accessions based on their geographical origin and habitats. This list includes 975 samples of *sativa* and *sylvestris* germplasm from France, the Iberian Peninsula, Georgia, Turkmenistan, Pakistan, Italy, and Croatia that were genotyped in previous studies by Laucou et al. [7], De Andrès et al. [8], Imazio et al. [12], Riaz et al. [24], Biagini et al. [28] and Zdunić et al. [29]. In this work, 403 new accessions of *V. vinifera* spp. *sylvestris* from Armenia, Azerbaijan, Georgia and Croatia were genotyped. The wild germplasm from Armenia, Azerbaijan, and Georgia was collected as seeds from female vines gathered on two different collection trips. Seedling plants from a total of 17 seed lots are maintained in the USDA National Clonal Germplasm Repository in Davis, California, USA. The *sylvestris* samples from Croatia were collected from plants located in their natural habitats mostly along the Krka and Neretva rivers in 2013. Care was taken to select plants that were dioecious and notes were made for the flower phenotype and leaf morphology [29]. The Spanish accessions collected from natural habitats are maintained in the "El Encín" germplasm repository (Madrid, Spain). The French *sylvestris* accessions are maintained in

Table 1 List of cultivated and wild accessions of *Vitis vinifera* (1378) grouped into countries based on their geographic origin and analyzed by 20 SSR markers. Number of samples for each country is presented in brackets

V. vinifera subsp. *sativa* (396)		*V. vinifera* subsp. *sylvestris* (982)	
Europe	Asia	Europe	Asia
Spain (145) [a]	Georgia (112) [d]	France (46) [c]	Armenia (49)
Italy (34) [b]	Turkmenistan and Pakistan (73)[e]	Italy (289) [b]	Azerbaijan (292)
France (32)[c]		Croatia (6) [f]	Georgia (46) [d]
		Croatia (32)	Georgia (30)
		Spain (192) [a]	
Total 211	185	565	417

[a][8]
[b][28]
[c][7]
[d][12]
[e][24]
[f][29]

the INRA "Domaine de Vassal" germplasm collection, and the Italian [28] and Georgian [12] samples are maintained in the germplasm repository of the University of Milan (Milano, Italy).

DNA extraction and genotyping

Total genomic DNA was extracted from young leaves using DNeasy Plant Mini Kits (Qiagen, Valencia, CA, USA). Genotyping was carried out by amplifying 20 nuclear SSR loci: VMC1b11, VMC4f3.1, VVIb01, VVIh54, VVIn16, VVIn73, VVIp31, VVIp60, VVIq52, VVIv37, VVIv67, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32, VVMD5, VVMD7, VVS2 [7]. The amplifications were performed as reported in [7]. The amplified loci were detected on an automated ABI 3500 Genetic Analyzer (Applied Biosystems, Life Technologies, Foster City, CA, USA). Allele sizes were scored using GeneMapper 4.0 software (Applied Biosystems, Life Technologies) and recorded in base pairs.

Data analysis

Determination of flower phenotype

The flower phenotype of the *V. vinifera* subsp. *sylvestris* samples collected from Armenia, Azerbaijan, Georgia and Croatia was determined by a combination of a specifically designed marker from gene APT3 (adenine phosphoribosyl transferase) that is capable of distinguishing female plants from males or hermaphrodites [24]. We also used a specific allele of the SSR marker VVIb23 that is closely linked with the sex locus on chromosome 2, and is capable of distinguishing hermaphrodites from female or male plants. The VVIb23 locus polymorphism has been detected and reported in [30]. A total of 403 accessions were analyzed with these two markers to assign flower phenotype. The flower phenotypes of additional wild accessions from other countries were determined either during the time of collection or from plants maintainted in the germplasm repositories.

Genetic diversity

In order to combine the fingerprint data of new genotypes with previous data sets [7, 8, 12, 24, 28, 29], genetic profiles of eight reference cultivars (Cabernet Sauvignon, Chardonnay, Dolcetto, Pinot noir, Riesling, Thompson Seedless, Zinfandel, and Sangiovese) were used as references to standardize the allele calls.

The genetic diversity among groups and over all the groups was estimated. The normalized SSR genotyping data were used to determine the number of different alleles (Na), the effective number of alleles (Ne), Shannon's Information Index (I), observed heterozygosity (Ho) and expected heterozygosity (He; [31]). The parameters were estimated by GenAlEx 6.5 software [32]. Weir and Cockerham's F-statistics (F_{IS}, F_{IT}, F_{ST}; [33]) per locus and F_{IS} values per each population were detected via FSTAT 2.9.3 and Arlequin 3.5.2.2 softwares, respectively [34, 35] *p*-values were evaluated over 1000 permutations. Allelic richness (AR) and private allelic richness (PAR) for each population were estimated using the rarefaction method, which compensates for differences in sample size (i.e. rarified allelic richness) among populations as implemented in HP-Rare 1.1 [36]. The effective number of migrants per generation (Nm) among the 12 grapevine populations and between the two subspecies was estimated using the private allele method of Barton and Slatkin [37] (1986) using GENEPOP 3.4 software [38].

Genetic relationships and differentiation

Poppr [39] package implemented in R 3.1 software [40] was used to design a phylogenetic tree with Neighbor-Joining. The distance matrix used in *Poppr* was calculated based on the Nei's distance [41]. The unrooted dendrogram was plotted with the R package *ape* [42]. To measure how well the hierarchical structure from the dendrogram represents the actual distances, the cophenetic correlation coefficient (CCC) has been calculated performing the cophenetic function implemented in R software. Hclust R function was used to perfume hierarchical clustering using a neighbor-joining agglomeration method. In order to elucidate the genetic relationships within and among geographic groups, principal coordinate analysis (PCoA) was performed on the multilocus microsatellite data, which was then arranged into geographic groups using the package *adegenet* implemented in R [43]. Clustering validation and multivariate analysis was carried out using pairwise Nei's genetic distance [44] and pairwise F_{ST} in GenAlEx 6.5 software. Finally, an analysis of molecular variance (AMOVA, [45]) was performed to characterize the partition of the observed genetic variation among and within populations and genetic groups using Arlequin 3.5.2.2 software. The significance test was performed over 1000 permutations.

Analysis of population structure

The microsatellite data were subjected to a Bayesian model-based cluster analysis using STRUCTURE 2.0 [46] to determine the optimum number of genetically supported groupings. STRUCTURE allocates individuals into a number of clusters (K) independent of population information based on genotypic data, so as to minimize deviations from Hardy-Weinberg and linkage equilibrium. The program uses a Markov Chain Monte Carlo (MCMC) procedure to estimate $P(X|K)$, the posterior probability that the data fit the hypothesis of K clusters. The analysis assigns individuals to each of the K clusters based on the membership coefficient (Q-value), which sums to unity over the number of clusters (K) assumed.

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STRUCTURE was set to ignore population information, and to use an admixture model with correlated allele frequencies as it is considered to be the best option for subtle population structure [47]. The degree of admixture, alpha, was allowed to be inferred from the data. Alpha is close to zero when most individuals are from one population or another, while alpha is greater than one when most individuals are admixed [48]. The allele frequency parameter (lambda) was set to one. During a pilot study, it was found that a burn-in and MCMC (Markov Chain Monte Carlo) simulation lengths of 100,000 replicate runs were optimum to produce accurate parameter estimates. The number of clusters (K) varied from 2 to 10, and 20 replicate runs were carried out to quantify the variation of the likelihood for each K. The K value that provides the maximum likelihood (Ln $P(D)$ in STRUCTURE) across runs is generally inferred as the most probable number of clusters. Nevertheless, the interpretation of K should be treated with care as it merely provides an ad hoc approximation [46] and sometimes genuine and fine population structure may be missed by STRUCTURE. Therefore, we used an ad hoc statistic ΔK to choose the optimum number of clusters (K) based on the second order rate of change in the log probability of data between successive K values as proposed by Evanno et al. [48].

Results

Flower phenotype in the wild accessions

Flower sex phenotype and seed morphology are key criteria normally used to differentiate subsp. *sylvestris* (dioecious vines, seeds with short beaks) from cultivated *sativa* forms (predominantly hermaphroditic flowers, seeds with larger beaks). The search for wild accessions was focused on collecting dioecious individuals because most cultivated genotypes are hermaphrodites. Flower phenotype data from the wild samples from Spain and Italy were recorded in the field and previously reported by Benito et al. [49] and Biagini et al. [28, 50]. The *sylvestris* samples from France, Georgia (University of Milan repository) and Croatia were collected from natural habitats and flower phenotypes were recorded based on the presence of fruit (female) and flower rachis without fruit (male) during collection. Only samples that met the basic dioecious phenotypic profile and leaf morphology of wild grapevines were included in the study. The flower phenotype of the subsp. *sylvestris* accessions collected from Armenia, Azerbaijan and Georgia (USDA repository) could not be determined because these plants were maintained in small containers. A combination of two DNA markers was used to differentiate the male, hermaphrodite and female flower phenotype for the set of 403 accessions from Armenia, Azerbaijan, Georgia and Croatia (Additional file 2: Table S2). Field phenotypic observations for the 38 accessions from Croatia matched the flower phenotype predicted by DNA analysis. Flower phenotypes assessed by DNA-based flower sex markers and field phenotyping of the wild forms of all the accessions of *V. vinifera* subsp. *sylvestris* are presented in Additional file 2: Table S2.

Genetic diversity for *sativa* and *sylvestris* germplasm

Genetic data from 20 SSR loci and across 1378 grapevine samples, originating from Asia to Europe (Table 1) and representing both subspecies of *V. vinifera* (*sativa* and *sylvestris*), were used in this study. Additional file 1: Table S1 provides the allelic profiles of all analyzed samples. The number of alleles ranged from 11 for VVIq52 to 38 for VMC4f3.1 with an average of 20.95 alleles/locus. The number of effective alleles ranged from 2.192 for VVIn73 to 7.004 for VVIp31 with an overall average of 4.651. Both observed and expected heterozygosity varied greatly among loci and results of the fixation index with most loci suggested high levels of inbreeding (Table 2). The He values ranged from 0.477 (VVIn73 locus) to 0.803 (VVS2), with a mean value equal to 0.678. While, the Ho values varied from 0.535 (VVIn73) to 0.845 (VVIp31) and the mean overall value was 0.742. The locus with the lowest F value was VVIb01 (0.021), while the highest was VVIq52 (0.189). The mean F value for the dataset was 0.088.

Allelic profiles were used to calculate statistical indices and determine the genetic diversity of the cultivated and wild genotypes (Table 3). The number of alleles per locus (Na) was 9.120 for *sativa* and 9.164 for *sylvestris* samples. The Italian cultivars had the lowest Na value (4.900) of the cultivated accessions and the highest Na value (12.600) was detected in the Georgian cultivars. The number of alleles per locus for the wild accessions varied between 7.050 (Armenia) and 12.850 (Georgia). The Ne value over the whole dataset was 4.441. The *sativa* accessions from Italy (3.688) and *sylvestris* accessions from France (2.792) had the lowest Ne values. The highest Ne values were detected in cultivated accessions (5.751) and wild individuals (6.016, Table 3) from Georgia. Within *sativa*, the allelic richness, adjusted to a minimum sample size of 42 genes, ranged from 6.200 alleles for Spanish accessions to 9.330 for Italian accessions, with an overall mean of 7.848 alleles across loci. Within the *sylvestris* accessions, allelic richness ranged from 5.870 for the Armenian group to 10.200 for the Georgian group with an overall mean of 7.089 across loci. The private allelic richness for *sativa* ranged from 0.020 for the Spanish and French groups to 0.520 for the Italian and Turkmenistan/Pakistani groups with an overall mean frequency of 0.314 alleles across loci. Within *sylvestris*, this richness ranged from 0.020 for the Azerbaijani accessions to 0.980 for Georgian wild grapes with an overall mean of 0.344 private alleles per locus.

Table 2 Diversity indices* calculated for 1378 distinct genotypes including *sativa* and *sylvestris* accessions from Asia to Europe

Locus	Na[a]	Ne[b]	He[c]	Ho[d]	F[e]
VMC1b11	22	5.159	0.631	0.779	0.183
VMC4f3.1	38	5.970	0.776	0.810	0.041
VVIb01	20	3.261	0.662	0.681	0.021
VVIh54	25	4.213	0.665	0.747	0.116
VVIn16	14	2.551	0.538	0.566	0.054
VVIn73	14	2.192	0.477	0.535	0.120
VVIp31	25	7.004	0.790	0.845	0.065
VVIp60	20	4.581	0.703	0.758	0.071
VVIq52	11	2.862	0.519	0.634	0.189
VVIv37	21	5.694	0.667	0.792	0.153
VVIv67	26	5.314	0.719	0.790	0.089
VVMD21	18	2.617	0.490	0.571	0.138
VVMD24	12	3.754	0.666	0.720	0.072
VVMD25	23	4.987	0.760	0.789	0.035
VVMD27	20	4.576	0.678	0.767	0.117
VVMD28	31	5.960	0.724	0.819	0.115
VVMD32	19	5.017	0.734	0.785	0.061
VVMD5	20	5.142	0.766	0.800	0.042
VVMD7	20	5.595	0.785	0.804	0.023
VVS2	20	6.576	0.803	0.839	0.045
Mean	20.950	4.651	0.678	0.742	0.088

*[a]No. of allele per locus
[b]No. of effective alleles
[c]Expected Heterozygosity
[d]Observed Heterozygosity
[e]Fixation Index

The mean Shannon's Information Index (I) value for the wild accessions was slightly lower than that for the cultivars (1.60 vs. 1.641), with an overall value of 1.619 (Table 3). In general, the Ho values were lower than He values for each group, except for cultivated samples from France (0.765 vs. 0.708) and Italy (0.798 vs. 0.682). The Ho value for *sativa* was higher than *sylvestris* (0.754 vs. 0.649), while the overall mean value (0.692) was more similar to the *sylvestris* value than the *sativa* value. The He value for *sativa* (0.735) was higher than the *sylvestris* value (0.722).

The samples were arranged in 12 groups based on their origin and subspecies, and F_{IS} values were calculated (Table 3). The values ranged from − 0.166 (Italian *sativa* samples) to 0.138 (Georgian *sylvestris* samples). The values for the *sylvestris* populations were generally higher than the *sativa* populations. Among the wild accessions, populations from Georgia and Spain had the highest F_{IS} values (0.138 and 0.131, respectively). The populations of cultivated accessions with the highest inbreeding coefficient were from France (0.057) and Georgia (0.066). The F_{IS} value over all loci and populations was 0.151 and the *sativa* value was lower than that for *sylvestris* (0.039 versus 0.169). Most of the F_{IS} values had a *p*-value lower than 0.1.

Cluster analysis

The neighbor-joining (NJ) cluster analysis based on the pair-wise distance matrix showed clear differentiation between the two subspecies (Fig. 1). A number of wild individuals clustered with the cultivated samples and vice versa. The dendrogram showed three main groups with cophenetic correlation coefficient (CCC) value of 0.75 (Fig. 1). The *sylvestris* accessions divided into two groups and *sativa* accessions formed a third major group. The first group of wild germplasm contained most of the Transcaucasian *sylvestris* accessions from Armenia (#1), Azerbaijan (#2) and Georgia (#5) and the second group consisted of the European wild accessions from Croatia (#3), France (#4), Italy (#6) and Spain (#7). The Spanish wild accessions were further split into two groups, one of them including the French wild samples (#4). There were two sub-groupings within the *sativa* cluster, one containing the French (#8), Italian (#10), Spanish (#11) and Turkmenistan-Pakistan samples (#12), and the other containing some of the Georgian samples (#9). Two additional minor clusters were identified, both containing Georgian samples. One of these contained the wild samples (#5) and the other both wild and cultivated samples (#5 and #9). The latter cluster also contained a small group of Italian cultivars (#10).

Population structure analysis and differentiation

In order to identify the structure of populations and the correlations among samples, two different analyses were performed. PCoA was based on the genetic distance matrix obtained by the SSR profiles. Projections of the PCoA were plotted in a 2-dimension scatter plot (Fig. 2). The PCoA 2D projection of the first two principal axes accounted for ~ 32% of the total molecular variation (Fig. 2). Significant differentiation between the two subspecies and the European and Transcaucasian *sylvestris* groups was observed. The *sylvestris* samples from Armenia (#1), Azerbaijan (#2) and Georgia (#5) were clearly differentiated from the rest of the *sativa* and *sylvestris* groups. The European *sylvestris* groups (#3, #4, #6 and #7) formed overlapping clusters, as did the accessions from Armenia (#1) and Georgia (#5). All five groups of *sativa* from Europe (#8, #10 and #11), Georgia (#9), Turkmenistan and Pakistan (#12) were closely associated. The *sativa* groups were closely associated with *sylvestris* accessions from Europe (#3, 4, 6, 7) and Transcaucasia (#1, 5), with the exception of the *sylvestris* accessions from Azerbaijan (#2). There was large variability within each of these groups and subspecies. The second method used to evaluate the relationship among genotypes was a clustering algorithm implemented in the program STRUCTURE. The Bayesian analysis results of genetic structure for the wild

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Table 3 Genetic diversity estimates in wild and cultivated grapevines for each analyzed population. Results are arranged based on the geographical origin and habitat

Populations	N[a]	Na[b]	Ne[c]	AR[d]	PAR[e]	I[f]	Ho[g]	He[h]	F_{IS}[i]
France	25.750	6.900	4.035	6.720	0.020	1.516	0.765	0.708	0.057 ***
Georgia	103.100	12.600	5.751	8.530	0.490	1.877	0.746	0.776	0.066 ***
Italy	6.600	4.900	3.688	9.330	0.520	1.349	0.798	0.682	-0.166
Spain	144.500	10.350	4.650	6.200	0.020	1.670	0.730	0.739	0.022 ***
Turkmenistan, Pakistan	71.000	10.850	5.290	8.460	0.520	1.793	0.723	0.768	0.053 ***
Overall *sativa*	70.190	9.120	4.682	7.848	0.314	1.641	0.754	0.735	0.039 ***
Armenia	47.150	7.050	3.967	5.870	0.100	1.506	0.676	0.718	-0.077
Azerbaijan	278.450	8.550	3.649	5.980	0.020	1.476	0.650	0.694	0.095 ***
Croatia	36.850	9.650	4.849	8.260	0.880	1.779	0.658	0.759	-0.038 ***
France	45.650	6.350	2.792	5.912	0.143	1.202	0.591	0.604	0.035 **
Georgia	73.800	12.850	6.016	10.200	0.980	1.999	0.653	0.815	0.138 ***
Italy	289.000	10.250	4.044	6.410	0.160	1.569	0.660	0.709	0.055 ***
Spain	192.000	9.450	4.556	6.990	0.130	1.686	0.655	0.755	0.131 ***
Overall *sylvestris*	137.557	9.164	4.268	7.089	0.345	1.602	0.649	0.722	0.169 ***
Overall Loci and Pops	109.488	9.146	4.441	7.405	0.332	1.619	0.692	0.727	0.151 ***

[a]No. of samples; [b]No. of alleles per locus; [c]No. of effective alleles; [d]Allelic Richness; [e]Private allele richness; [f]Shannon's Information Index; [g]Observed heterozygosity; [h]Expected heterozygosity, [i]Inbreeding coefficient within individuals relative to the subpopulation; $^{**}p \leq 0.10$; $^{***}p \leq 0.05$ calculated over 1000 permutations

(*sylvestris*) and cultivated grapevines (*sativa*) were roughly comparable with the NJ cluster analysis and PCoA results, but STRUCTURE did not detect subtle differentiation among some of the populations. The estimated log probability values [Ln Pr (X|K)] for different K gradually increased reaching a maximum value at K = 3 with non-significant variation among replicate runs, beyond which the rate of increase between successive K decreased and variance among runs increased (Fig. 3). Plotting the second order rate of change of the log probability of data (ΔK) with respect to the number of clusters, against K predicts the true K according to Evanno et al. [48], and such analysis produced a clear peak at K = 2, but the second order rate of change of likelihood distribution showed that the rate of change is bigger between K = 3 and 4, therefore, K = 3 is the most likely number of clusters in the genetic structure of these grape populations. About 84% of genotypes were assigned to a cluster at K = 3, with a percentage

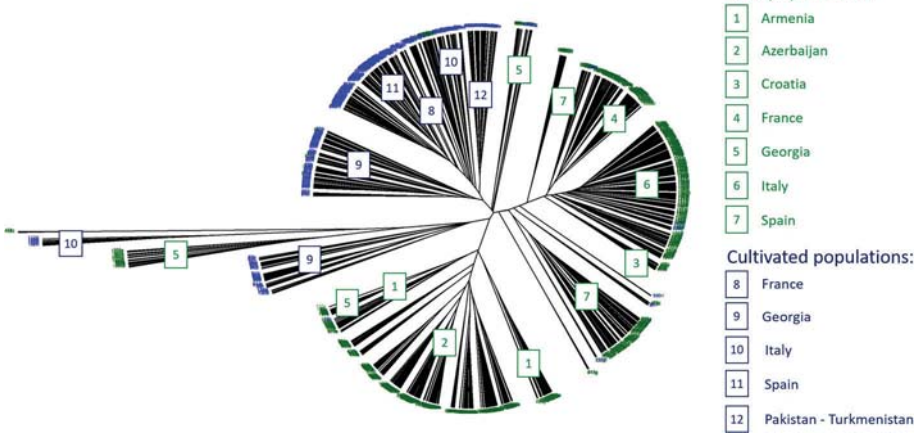

Fig. 1 NJ dendrogram showing relationships among 1378 cultivated and wild grapevine accessions obtained by data analysis from 20 SSR loci. Samples are arranged based on their origin and membership in the sativa and sylvestris subspecies

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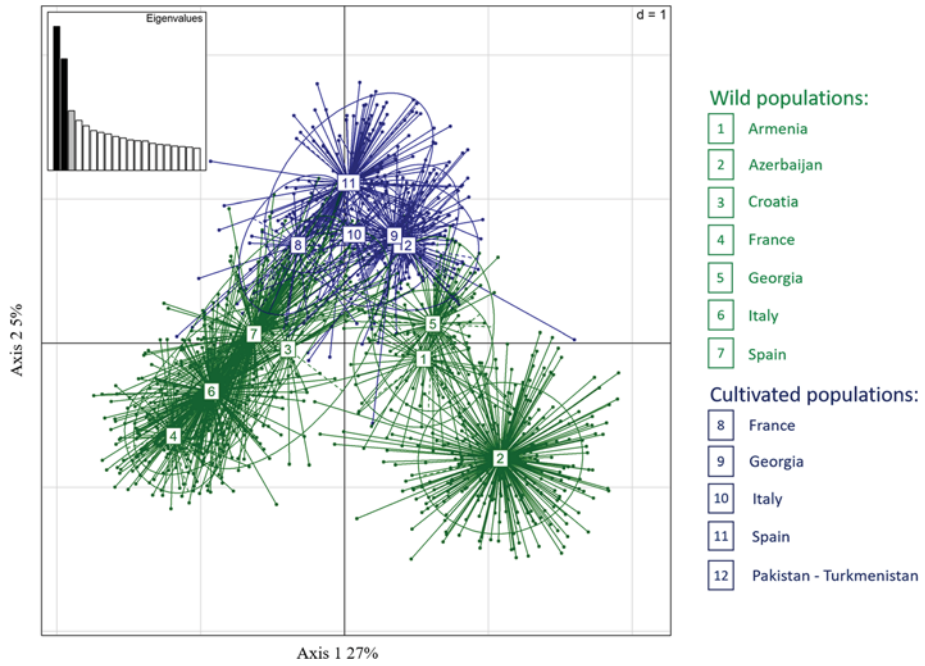

Fig. 2 Relationships between wild and cultivated grapevine genotypes (1378) as represented by the first two principal coordinates of a PCoA using allelic profiles from 20 SSR molecular markers. Samples are arranged based on their origin and membership in the sativa and sylvestris subspecies

of assignment higher than 80%. The proportion of admixed genotypes was about 16% (Additional file 3: Table S3). Plotting the Q matrix values (the estimated membership coefficients for each individual in each K clusters) for K = 3 (Fig. 3), revealed clusters roughly corresponding to the two major groups within *sylvestris*, one from the Caucasus (Armenia, Azerbaijan and Georgia; G2) and the other from Europe (Croatia, France, Italy and Spain; G3), and one group with the French, Georgian, Italian and Spanish *sativa* accessions (G1). As observed in the NJ cluster analysis and PCoA, there were genotypes with mixed ancestry in all three groups. The populations with the highest percentage of admixed samples were Armenia (39%) and Georgia (49%) for wild groups and France (32%) for sativa accessions (Additional file 3: Table S3).

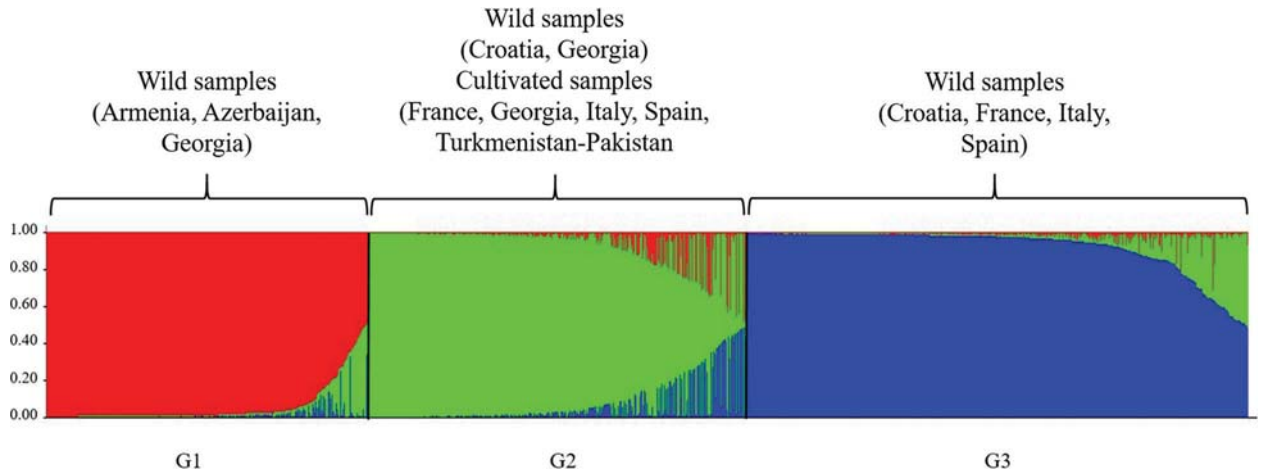

Fig. 3 Barplot displaying the admixture proportions of wild and cultivated grapevine genotypes as estimated by STRUCTURE analysis at K = 3 and 7. The Evanno's ΔKs statistics indicated K = 3 as the best supported level of population subdivision using simulation model with K values ranging from 2 to 10

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Population structure among the 12 tested populations, irrespective of the subspecies, was summarized by the Wright's F-statistics (F_{IT}, F_{ST} and F_{IS}) (Additional file 4: Table S4). The VVMD21 locus had the highest value for F_{IT}, F_{ST} and F_{IS} (0.380, 0.235 and 0.189, respectively), while the lowest F_{IT} and F_{IS} values were detected for the VMC4f3.1 locus (0.095 and 0.005, respectively), and VVMD25 had the lowest F_{ST} value (0.056). The number of migrants (Nm) after correction for sample size was 1.33, when samples were arranged in 12 populations. When the samples were arranged in two subpopulations (*sativa* and *sylvestris*), Nm was 4.88.

Nei's genetic distance and F_{ST} were calculated to validate the results obtained from cluster analysis and PCoA. The pairwise values for the 12 geographic groups are listed in Table 4. Nei's genetic distance had a wide range of values, from 0.116 recorded for the pairwise French and Spanish *sativa* samples, to 0.830 for the *sylvestris* samples from Georgia and France. The F_{ST} values varied from a low of 0.021 detected for the French and Spanish cultivated accessions to a high of 0.125 for the *sylvestris* individuals from Azerbaijan and France. Nei's genetic distance and F_{ST} values for *sativa* and *sylvestris* groups were 0.159 and 0.023, respectively.

The AMOVA analysis is presented in Additional file 5: Table S5. When the total genetic variation was partitioned, 9.54% was attributed to the differences among populations, 6.68% to the differences among individuals within populations and 83.78% to the differences within individuals, with levels of significance estimated over 1000 permutations lower than 0.05. F_{ST}, F_{IS} and F_{IT} parameters overall the loci and populations were 0.095, 0.073 and 0.162, respectively ($p \leq 0.05$).

Discussion

The main objective of this study was to analyze the pattern of genetic diversity within and between wild and cultivated grapes from the Mediterranean basin and Central Asia – considered to be the center of domestication. We pooled information from six previous studies that examined both wild and cultivated accessions, and genotyped an additiopnal 403 wild accessions from the Caucases region and Croatia at 20 microsatellite loci. The microsatellite marker data from 1378 accessions was subjected to NJ clustering and Bayesian methods to elucidate groupings of wild grapevine populations and to infer gene flow and gene frequency changes that occurred during domestication.

Assessment of flower sex within *sylvestris* populations

Taxonomic distinctions between the two subspecies, *sylvestris* and *sativa*, are based on leaf morphology and the dioecious state of wild forms. According to the model of Antcliff [51], the flower phenotype is controlled by a single major locus with three alleles: male (M) dominant to hermaphrodite (H), which is dominant to the female (F). In the wild, only male and female vines exist in the absence of gene flow from hermaphroditic cultivated varieties. However, the possibility of hybridization and seed dispersion increases where wild vines are in close proximity to cultivated types. The wild accessions from earlier studies were collected with careful consideration of flower phenotype and leaf morphology [7, 8, 12, 28, 29]. The samples from Armenia, Azerbaijan, and Georgia were collected as seed lots. Analyses of flower phenotype based on linked markers found that the Georgia populations had more female than male vines, and that seed lot DVIT3357 consisted of only

Table 4 Estimates of pairwise Nei's genetic distance (below the diagonal) and F_{ST} values (above the diagonal) within overall wild and cultivated grapevine groups

	Armenia	Azerbaijan	Croatia	France (*sylvestris*)	France (*sativa*)	Georgia (*sylvestris*)	Georgia (*sativa*)	Italy (*sylvestris*)	Italy (*sativa*)	Spain (*sylvestris*)	Spain (*sativa*)	Turkmenistan, Pakistan
Armenia	–	**0.043**	**0.173**	**0.208**	**0.172**	**0.097**	**0.106**	**0.139**	**0.160**	**0.123**	**0.144**	**0.131**
Azerbaijan	0.268	**–**	**0.120**	**0.177**	**0.146**	**0.062**	**0.064**	**0.117**	**0.115**	**0.088**	**0.104**	**0.091**
Croatia	0.457	0.463	–	**0.189**	**0.164**	**0.086**	**0.069**	**0.116**	**0.076**	**0.003**	**0.061**	**0.067**
France (*sylvestris*)	0.721	0.730	0.363	–	**0.101**	**0.198**	**0.175**	**0.101**	**0.184**	**0.122**	**0.154**	**0.196**
France (*sativa*)	0.439	0.603	0.290	0.473	–	**0.175**	**0.141**	**0.084**	**0.150**	**0.080**	**0.128**	**0.180**
Georgia (*sylvestris*)	0.254	0.243	0.458	0.830	0.423	–	**0.054**	**0.108**	**0.096**	**0.085**	**0.079**	**0.061**
Georgia (*sativa*)	0.465	0.515	0.421	0.830	0.295	0.269	–	**0.100**	**0.050**	**0.049**	**0.058**	**0.050**
Italy (*sylvestris*)	0.409	0.533	0.213	0.262	0.291	0.469	0.471	–	**0.117**	**0.078**	**0.094**	**0.116**
Italy (*sativa*)	0.575	0.702	0.432	0.748	0.312	0.478	0.288	0.470	–	**0.066**	**0.066**	**0.066**
Spain (*sylvestris*)	0.576	0.565	0.289	0.286	0.303	0.544	0.502	0.247	0.501	–	**0.044**	**0.071**
Spain (*sativa*)	0.419	0.629	0.384	0.686	0.116	0.396	0.261	0.427	0.253	0.359	–	**0.056**
Turkmenistan, Pakistan	0.322	0.484	0.448	0.774	0.327	0.353	0.278	0.467	0.338	0.510	0.253	–

In bold, significant values with $p \leq 0.05$, calculated over 1000 permutations

female and hermaphrodite vines indicating gene flow from cultivated to wild types (Additional file 2: Table S2). However, the Armenian, and Azerbaijan populations had a higher proportion of male plants. Heterogeneous plant sex distribution was also observed in earlier study of Spanish *sylvestris* samples [49] with a majority of the plants being male.

Pattern of genetic diversity distribution within and among the subspecies

The two subspecies of *V. vinifera* included in this study exhibited high levels of polymorphism and heterozygosity across the 20 microsatellite loci and significant diversity was observed within and between the subspecies (Tables 2 and 3). This trend was expected in a divergent gene pool composed of subspecies and hermaphroditic cultivars that have undergone intensive human selection during domestication. Data obtained in other studies [10, 11] are similar to the results from our survey. Genetic diversity within and among the different geographic groups in both subspecies, as demonstrated by the effective number of alleles and allelic richness, suggests that there is significant diversity both within and between the subspecies (Table 3). The *sativa* and *sylvestris* accessions from Georgia had the highest number of effective alleles and allele richness suggesting that this region is the center of diversity for *V. vinifera* [2].

In general, we expected to see higher levels of heterozygosity in *sylvestris* because of its obligate out-crossing nature compared to its domesticated counterpart *sativa*. The Ho value of the *sativa* group appeared slightly higher than the He values; while the trend was the opposite for the *sylvestris* accessions. These differences correspond with the positive F_{IS} values in *sylvestris*, particularly in the populations from Spain and Georgia, which suggests a high level of genetic relationship among the individuals from the same wild populations (Table 3). Such matings can affect individual and population dynamics and increase inbreeding. However, the F_{IS} values of some wild populations were close to zero as expected in randomly mating populations (Table 3). These opposing results may be explained by random genetic drift of alleles among subpopulations due to sample size. The reduced level of diversity that we observed in *sylvestris* samples has also been noted in other studies [10–12]. The *sylvestris* accessions in many parts of the world are considered endangered and fragmented due to deforestation and urbanization. Man-made and natural geographical barriers can also lead to the isolation of wild populations in their native habitat, and could lead to significant inbreeding, reduced gene flow within and among different geographic groups and, hence, lower levels of heterozygosity.

The F_{IS} values were close to zero in the cultivated accessions suggesting random mating, except the Italian accessions. The negative F_{IS} values for Italian populations indicated an excess of heterozygotes, but it was not statistically significant (Table 3). The deficiency of homozygotes in the majority of the cultivated groupings suggests that they are made up of germplasm with divergent demographic (founder effects, bottlenecks, dispersal) and selection histories. Germplasm collections are usually mixtures of genotypes. Thus, geographic groups in these collections exhibit relatively high levels of differentiation, resulting in higher than expected levels of heterozygosity. This is commonly observed in woody perennial crops where cultivars are selected for their vigor, which indirectly favors high levels of heterozygosity [52–54].

The results of the AMOVA and F-stat analysis confirmed that high levels of diversity were present within populations, while low levels of genetic diversity were found among populations. These results are consistent with the findings from other studies [10–12].

Genetic structure and differentiation within and between the subspecies

A significant differentiation within and between the two subspecies was detected by cluster analysis and PCoA (Figs. 1 and 2). Both analyses found clear differentiation between the Western European wild grapevines and the wild samples collected from the Caucasus. The French and Spanish wild grapes were closely allied and had a close genetic relationship. These results were in agreement with Arroyo et al. [22], who used chloroplast markers to find that these populations had the same haplotype. The Spanish wild grapevines showed hierarchical differentiation, suggesting that gene flow among neighboring populations caused a stepping-stone model of population structure. Alternatively, the hierarchical differentiation could be the result of climatic differences across diverse geographic regions. The Croatian *sylvestris* accessions were related to the European *sylvestris* individuals and formed a basal sister group indicating a common gene pool. The wild grapevines from Transcaucasia, including Armenia, Azerbaijan, and Georgia, formed a distinct sub-group that contained several accessions of Azerbaijani wild grapevines. Similarly, the Georgian and Armenian wild grapes split into two subgroups each, however they shared a common Transcaucasia gene pool. The *sylvestris* vines in the Transcaucasia region grow in a wide range of isolated habitats created by the Greater and Lesser Caucasus Mountain systems where they are differentially adapted to local environments [12, 54]. Some of the *sylvestris* individuals, both in Caucasian and

European germplasm, clustered with the cultivated samples. These accessions are most likely feral hybrids of *sativa* and *sylvestris*, which may have been used in breeding programs or as cultivated selections (Figs 1 and 2).

Within *sativa*, two distinct groups of cultivars from Georgia were observed, one appeared as a sister clade of Italian, French and Spanish cultivars (Fig. 1), while the other group was closely related to an Italian *sativa* and Georgian *sylvestris* sub-group. This result could suggest that the first domesticated cultivars in Central Asia and Caucasus (*proles pontica*), left a genetic footprint in the Western European *proles occidentalis* accessions. This genetic kinship could also be a reflection of early breeding programs in the Mediterranean region where *sylvestris* or hybrid feral vines with superior fruit were utilized in crosses with domesticated lines.

The overall pattern of differentiation depicted by the PCoA is very similar to the NJ cluster analysis (Figs. 1 and 2). Clusters within *sylvestris* accessions from Georgia and Armenia overlapped and were closely associated with cultivated forms from Georgia, Pakistan and Turkmenistan. The close association of Georgian wild grapevines with Georgian cultivated accessions strongly supports their involvement in the initial domestication of grapevine [55–57]. Evaluation with NJ cluster analysis and PCoA, indicates that local European *sylvestris* vines might have contributed to the selection and introgression of genes into Western European grapevines in the later part of the domestication process (Fig. 2). The Bayesian STRUCTURE analysis supported differentiation among the major groups only, while the fine-scale differentiation between some of the groups, especially those with mixed ancestry, was not evident (Fig. 3). Bayesian inference of genetic structure indicated considerable gene flow with moderate differentiation between the two subspecies. These results suggest that wine grape cultivation and wine making promoted the domestication of wild grapevines, creation of new varieties, and advancement of growing techniques early in grapevine's history. Further introgression and mixing of wild germplasm in localized communities would have contributed to the high proportion of grapevines with mixed ancestry. Interestingly, analyses of ancestry values of tested western cultivars identify some with a high ancestry values in Group 3 (Additional file 3: Table S3). These grapevine cultivars correspond to the Spanish cultivars; Albariño, Caiño Blanco, Ferrón, Maturana, Ondarrabi Betlza and the European cultivars Arvine Petite, Cot, Chenin Blanc, Petit Verdot. Pinot Meunier and Sauvignon Blanc. These cultivars have been described as more closely related to wild accessions [8] and our results support the introgression of western *sylvestris* into some of the current Western European cultivars.

It is difficult to suggest that wild grape forms homogeneous populations considering the vast geographic expanse and the often fragmented and isolated populations that occur under heterogeneous climatic conditions. However, our results suggest Georgia as an ancient center of grapevine domestication with its wild grapes closely related to the cultivated grapes of the same region (*proles pontica*), and Western European (*proles occidentalis*). This observation confirms earlier studies that suggested that *proles pontica* were gradually introduced by human migration towards Western Europe [10, 25, 58, 59]. Cluster analysis shows a relationship between Western European wild grapes and cultivated grapes, suggesting that *proles occidentalis* grapevines contributed to the early development of wine grapes to a much greater extent than the wild vines from Eastern Europe. Previous studies using SNPs markers [25] proposed a Near East origin of *vinifera* and presented evidence of introgression from local *sylvestris* as the grape moved into Europe, but the degree to which local Western European wild *sylvestris* genetically contributed to Western European *vinifera* cultivars remains a contentious issue. Our results suggest and support at least two separate domestication events that gave raise to cultivated grape; one derived from the Transcaucasia wild grape, and another from the wild grapes of Western Europe.

Scientific interest in the highly endangered ancestor of cultivated grapevine, *V. vinifera* subsp. *sylvestris*, has centered on questions of conservation genetics, and deepening our understanding of the domestication history of the cultivated crop [22]. However, since domestication traits such as higher yield, larger berries, higher sugar content are often accompanied by a loss of resistance to abiotic and biotic stress, it is beneficial to search for such factors in the wild forms of the crop's ancestors. In fact, salt-tolerant grape accessions can be found in the North African *sylvestris* population [60], and the recent identification of wild and cultivated accessions from Germany, Iran and Georgia with tolerance to mildew diseases supports the potential of this wild ancestor as a genetic resource for disease resistance breeding [24, 61–63]. Given that wild Eurasian and North Africa wild *V. vinifera* germplasm and Asian *Vitis* germplasm are largely unexplored, their identification, preservation, and characterization for biotic and abiotic resistance and berry quality [64, 65] traits are very important for the future of the wine and grape industry.

Conclusions

The two sub-species of *V. vinifera*, subsp. *sativa* and subsp. *sylvestris*, are distinct based on analysis of SSR data, but extensive gene flow was observed in regions where these two taxa came in contact. Our results suggest that Georgia is an ancient center of grape domestication based on a genetic affinity between wild accessions from Georgia and cultivated grapes from Georgia (*pontica*) and Western

Europe (*occidentalis*). Results also suggest that Western European wild grapes were related to cultivated grapes, and that Western European *sylvestris* contributed to the development of Western European wine grapes. The re-sults also support at least two separate domestication events that gave raise to cultivated grape; one derived from the Transcaucasian wild grape and another from the wild grape of Western Europe.

Finally, wild grape germplasm can contribute many useful traits such as resistance to damaging pests and diseases, and better adaptation to climate change. Thus, we must intensify efforts to collect, characterize and preserve not only the Western and Eurasian wild *V. vinifera* germplasm, but also *sylvestris* accessions from North Africa and Central Asia. These wild grape relatives will have a key role in future grape and wine industry.

Additional files

Additional file 1: Table S1. SSR allelic profile of 1378 sativa and sylvestris grapevine samples genotyped at 20 SSR molecular markers. (XLSX 643 kb)

Additional file 2: Table S2. Determination of flower phenotype and genotype. The APT3 gene marker distinguished females (F) from males (M) or hermaphrodites (H). SSR marker VVIb23 is tightly linked to the flower sex locus and the unique allele 282 is linked to hermaphrodism. (XLSX 57 kb)

Additional file 3: Table S3. Ancestry values (mean and standard deviation values over 20 iterations) for the genetic groups inferred by STRUCTURE analysis of 1378 sativa and sylvestris grapevine samples genotyped at 20 SSR loci. (XLSX 145 kb)

Additional file 4: Table S4. Locus-wise genetic differentiation parameter comparisons of 12 populations from both *V. vinifera* subspecies sativa and sylvestris. (DOCX 19 kb)

Additional file 5: Table S5. Results of the AMOVA analysis carried out among and within 12 populations of wild and cultivated grapevine. (DOCX 16 kb)

Abbreviations

AMOVA: Analysis molecular of variance; BCE: Before current era; CE: Current era; F: Female; F: Inbreeding coefficient; Fst: Fixation index; H: Hermaphrodite; He: Expected heterozygosity; Ho: Observed heterozygosity; M: Male; N: Sample size; Ne: Number of effective alleles; PCoA: Principal coordinate analysis; SNP: Single Nucleotide Polymorphism; SSR: Simple sequence repeat; UMIL: University of Milan; USDA: United States Department of Agriculture

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Ethics approval and consent participate

Not applicable

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Availability of data and materials

A table of all genotypes and SSR allele calls used for statistical analysis in this study is submitted as Additional file 1: Table S1.

Authors' contributions

RA conceived the idea and design of the study and wrote the manuscript; SR and GDL developed fingerprint data, carried out genetic population analysis and assisted with drafting the manuscript, AK assisted in developing fingerprint profiles; MA assisted in the interpretation of the genetic diversity and population analysis and participated in the drafting of the manuscript; GZ provided the Croatian grape germplasm fingerprint database, DM, ZB and MM provided the Georgian germplasm; MAW, OF and JP were involved in discussion and interpretation of the results and oversaw the final draft and revisions. All authors have read and approved the final manuscript.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Study of Genetic Diversity in *V. vinifera* subsp. *sylvestris* in Azerbaijan and Georgia and Relationship with Species of the Cultivated Compartment

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Keywords: Caucasus, EU-COST project, genotyping, germplasm, SSR

Abstract

***Vitis vinifera* subsp. *sylvestris* is the wild progenitor of cultivated grapevine (*V. vinifera* subsp. *sativa*). Wild grapevine populations are part of the Eurasian flora, from Central Asia to the Mediterranean Basin. In most countries, the wild grapevine is considered on the brink of extinction and for this reason several research projects around the world – and particularly in Europe – are aimed to study the genetic diversity of wild grapevines in order to set up a germplasm collection. The recovery and characterization of wild grapevine genetic resources in the Caucasian region, which is considered to be the birth place for viticulture, is one of the main goals of the COST Action FA1003 European project. In the frame of this project, wild grapevine samples collected in Azerbaijan and Georgia were analysed with nine SSR molecular markers. From these molecular data, the genetic relationship of this wild material with local cultivars as well as the wild and cultivated compartments of European germplasm were evaluated. The SSR allelic patterns were analysed with GenAlEx 4.2 software to investigate the genetic diversity and STRUCTURE 3.2 software to investigate the admixture proportions among germplasms. By PCoA (Principal Coordinates Analysis) of which the two first coordinates accounted for about 50% of total variability, genotypes were classified in two main groups: i) European and Georgian germplasm; ii) Azerbaijani germplasm. STRUCTURE analysis revealed clearly the diversity of European germplasm in respect to the Caucasian germplasm. In conclusion, the analysis of genetic relationships in our dataset provided evidence of gene flow between wild and cultivated genotypes in the Caucasian germplasm.**

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most widely cultivated species of agricultural interest (Vivier and Pretorius, 2002). Its distribution area extends from Central Asia to the Mediterranean Basin (Zohary and Hopf, 2000). Two subspecies of *V. vinifera*, subsp. *sylvestris* and subsp. *sativa*, are considered to co-exist in the modern world. The first one is represented by wild populations and the second one is represented by cultivated varieties. It is usually acknowledged that cultivated varieties are originating from a wild pool of individuals that encountered domestication. The two subspecies show differences in several phenotypic traits, but one of the most distinctive traits among those is the sex determinism – dioecious in wild grapes and hermaphroditic, or to a lesser extent female, uncultivated grapes (Zohary, 1995).

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Because the subsp. *sylvestris* is considered to be the progenitor of subsp. *sativa*, many researchers focused their activities on the genetic investigation of the wild compartment and its relationship with cultivated individuals (De Andrès et al., 2011; Myles et al., 2011; Bacilieri et al., 2013; Imazio et al., 2013). It is estimated that the domestication of wild grapes started in the Neolithic Age, about 8,000 years ago, as a result of a long and gradual process closely linked to winemaking (This et al., 2006; Forni, 2012). Archaeological remains and proto-historical sources suggest the Near East area, comprising Oriental Anatolia, Syria, South Caucasus and the area around northern Mesopotamia, as the first center of domestication (McGovern, 2003; Forni, 2012).

The exploration of wild germplasm in the Caucasian region and its genetic and phenotypic characterization is one of the main focuses of the EU-COST project entitled "East-West collaboration for grapevine diversity exploration and mobilization of adaptive traits for breeding" (COST Action FA1003). In the frame of this project, autochthonous wild and cultivated individuals of Azerbaijan and Georgia (the South Caucasus region) were genotyped with nuclear SSR (Simple Sequence Repeat) molecular markers and the genetic relationships among Caucasian samples and the wild and cultivated compartments of European germplasm were investigated. SSR markers were selected in this study among many other molecular markers because they present the advantages of being already extensively used to determine genetic diversity in cultivated *V. vinifera* (Bacilieri et al., 2013), of solving cases of homonyms and synonyms (Laucou et al., 2011) and they allow to establish pedigree analysis (De Lorenzis et al., 2012).

MATERIALS AND METHODS

One hundred and nine cultivated and wild grapevine samples originating from Azerbaijan and Georgia were analyzed in this work (Table 1). The cultivated samples, representative of autochthonous germplasm of Azerbaijan and Georgia, were collected by the repositories (grapevine collection of Research Institute of Viticulture and Winemaking, Ministry of Agriculture, Baku, and the Skra collection (GEO015), located in the Skra Testing Station of the Institute of Horticulture, Viticulture and Oenology, Gori district in Kartli).

About the wild individuals, the Azerbaijani samples were collected in the Quba region, while the Georgian accessions were collected in Kakheti and Kartli provinces.

Fifty among European varieties (the most important international cultivars, collected in the Riccagioia germplasm collection – Italy) and wild individuals (a selection of the Italian wild accessions analyzed in Biagini et al., 2014) were included as out-group. Extraction of DNA was performed with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) starting from 0.02 g of dried young leaves. The samples were genotyped with nine microsatellite (SSR) markers: VrZag62, VrZag79, VVMD5, VVMD7, VVMD27, VVMD28, VVMD25, VVMD32, VVS2 (Laucou et al., 2011). Multiplexed PCR amplifications were performed in a 25-µl final volume reaction mixture following the method described in De Lorenzis et al. (2012).

The structure of populations was investigated by Principal Coordinates Analysis PCA) using GenAlEx 6.2 software (Peakall and Smouse, 2006) and STRUCTURE analysis (STRUCTURE 3.2 software; Pritchard et al., 2000). K value (K number of ancestral genetic groups) was chosen according to Evanno et al. (2005) method.

RESULTS AND DISCUSSION

A total of 159 *sativa* and *sylvestris* genotypes were collected in Azerbaijan, Georgia and Europe. The cultivated samples come from germplasm collections located in each country and are representative of the local variability, while the wild samples were collected in Quba region (Azerbaijan) and Kakheti and Kartli provinces (Georgia). The genotypes of both cultivated and wild samples coming from Caucasus were compared with 22 allelic profiles of international grapevine cultivars and 28 Italian wild accessions. They were analyzed with 9 SSR loci, in order to evaluate the correlation between cultivated and wild compartments in Caucasus and its relationship with European germplasm. Two different methods were performed. The first one, the Principal Component Analysis (PCA), is based on the genetic distance matrix obtained by SSR patterns. The two dimensional projections of PCA per each sample

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were plotted in a 2-D scattered plot (Fig. 1). The two first coordinates accounted for 29.04 and 23.94% of the total variation. The PCA scatter plot differentiated the samples into two main clusters: i) a first group containing most of the Azerbaijani individuals, including both *sativa* and *sylvestris* subspecies, ii) the second group comprised Georgian and European samples. Nevertheless, overlapping zones between the two main groups were observed and are highlighted in Figure 1. In the second cluster, we clearly identified an east-to-west distribution from Caucasus, considered as the first centre of grapevine domestication, to Europe, as previously described by Myles et al. (2011) and subsequently confirmed by Imazio et al. (2013). The differentiation of Azerbaijani from Georgian or European germplasm could be also linked to historical and cultural events. Indeed, Azerbaijan is a Muslim country while Georgia or European countries are Christian explaining the prevalence of table grape production in Azerbaijan area instead of wine and, in consequence, different *Vitis* genetic backgrounds. In this dataset, cultivated and wild compartments were not clearly differentiated, confirming a close relationship between *sativa* and *sylvestris* individuals and a gene flow between cultivated and wild compartments (De Andres et al., 2012; Imazio et al., 2013; Myles et al., 2011).

The second method of analysis is commonly used to infer the relationships among genotypes and is based on clustering algorithms implemented in the STRUCTURE program. The country of individuals was used as prior information. In order to uncover the hierarchical population structure, different numbers of K populations (number of clusters characterized by a set of allele frequenciesat each locus) were explored (Fig. 2). Optimal K (Evanno et al., 2005) estimated the most likely number of populations at K=3. Using a >80% threshold for group assignation, about 79% of samples were assigned to a cluster at K=3. The other 21% were admixed genotypes. The STRUCTURE analysis highlighted 3 groups arranged based on their geographical origin: i) Azerbaijani germplasm; ii) Georgian germplasm; iii) European germplasm. The identification of three different groups was in agreement with the classification in proles (*occidentalis*, *pontica*, *orientalis*) proposed by Negrul (1946). The percentage of admixed genotypes, especially in the Azerbaijani and Georgian germplasm, highlighted the originality and heterozygosity of Caucasian germplasm in respect to the European accessions.

CONCLUSIONS

This work confirmed the usefulness of highly polymorphic SSR markers to improve information on genetic diversity and genetic relationship among wild and cultivated compartments growing in different geographical areas, i.e., Caucasus and Europe. According to our results, we confirmed a gene flow between wild and cultivated grapevines and a genetic contribution of wild accessions to current cultivated varieties can be assumed. Moreover, the differentiation appears to reflect the pathway of grapevine dissemination from east towards west.

ACKNOWLEDGEMENTS

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Tables

Table 1. Number and origin ofcultivated and wild grapevine samples analyzed in this work.

Country	*V. vinifera* subsp. *sativa*	*V. vinifera* subsp. *sylvestris*	Total
Azerbaijan	38	4	42
Georgia	44	23	67
Europe	22	28	50
Total	104	55	159

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Figures

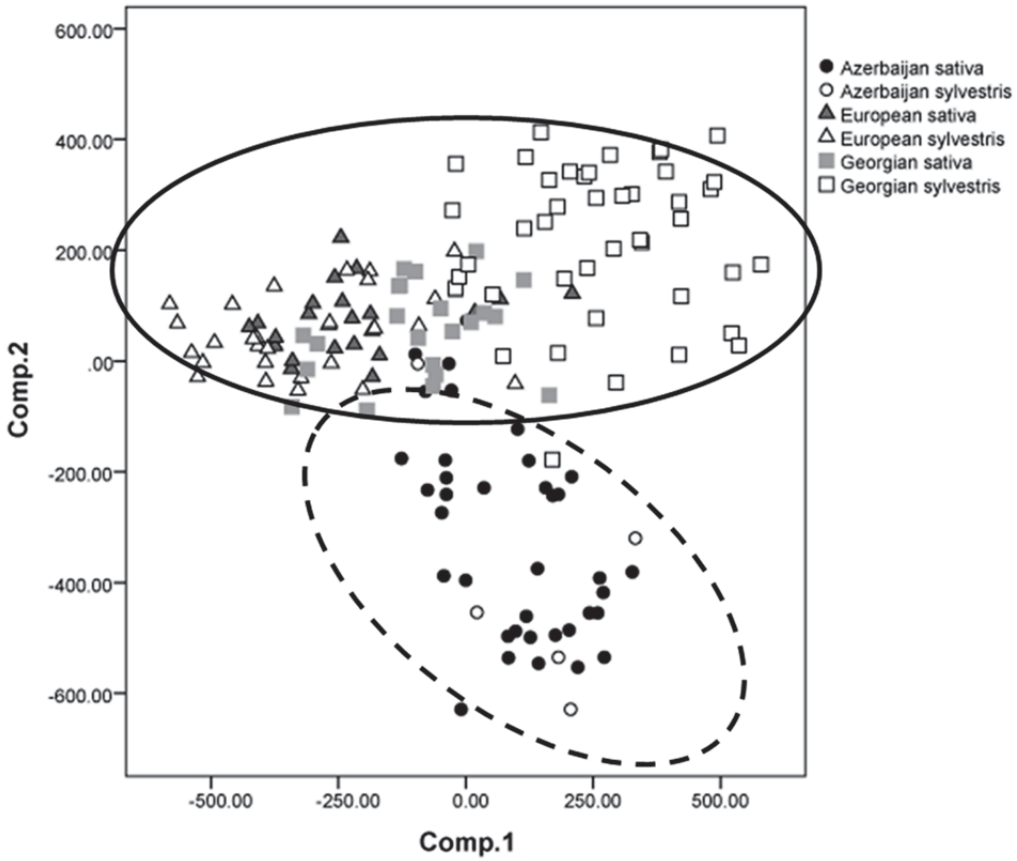

Fig. 1. Relationships between 159 cultivated and wild samples from Azerbaijan, Georgia and Europe as represented by the first two principal coordinates of PCA detected by SSR data. Solid line: Georgian and European samples; Dotted line: Azerbaijani samples.

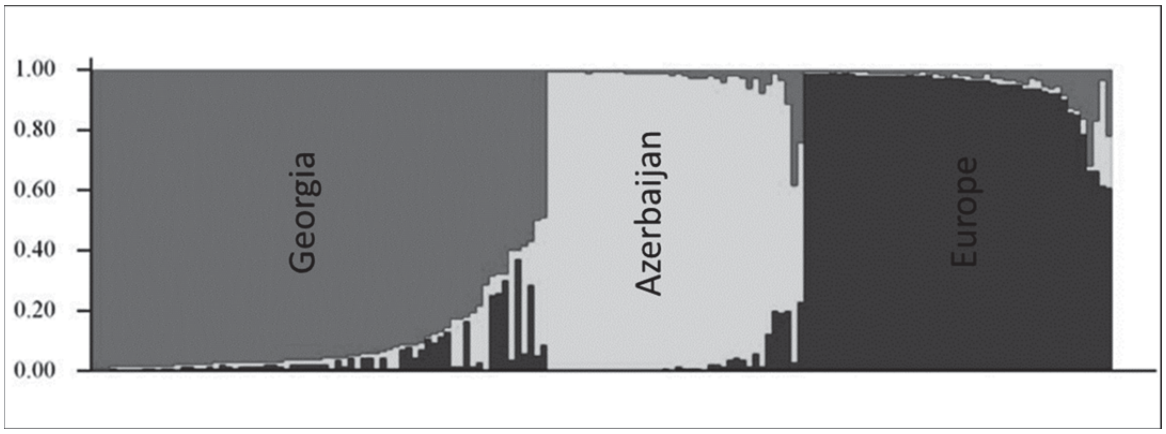

Fig. 2. Admixture proportions of 159 cultivated and wild samples from Azerbaijan, Georgia and Europe as estimated by STRUCTURE at K=3, displayed in a barplot. Each sample is represented as a vertical bar, reflecting assignment probabilities to each of the three groups.

Chapter 6

Genetics of Traits

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BMC Plant Biology

RESEARCH ARTICLE Open Access

A small XY chromosomal region explains sex determination in wild dioecious *V. vinifera* and the reversal to hermaphroditism in domesticated grapevines

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Abstract

Background: In *Vitis vinifera* L., domestication induced a dramatic change in flower morphology: the wild *sylvestris* subspecies is dioecious while hermaphroditism is largely predominant in the domesticated subsp. *V. v. vinifera*. The characterisation of polymorphisms in genes underlying the sex-determining chromosomal region may help clarify the history of domestication in grapevine and the evolution of sex chromosomes in plants. In the genus *Vitis*, sex determination is putatively controlled by one major locus with three alleles, male *M*, hermaphrodite *H* and female *F*, with an allelic dominance $M > H > F$. Previous genetic studies located the sex locus on chromosome 2. We used DNA polymorphisms of geographically diverse *V. vinifera* genotypes to confirm the position of this locus, to characterise the genetic diversity and traces of selection in candidate genes, and to explore the origin of hermaphroditism.

Results: In *V. v. sylvestris*, a sex-determining region of 154.8 kb, also present in other *Vitis* species, spans less than 1% of chromosome 2. It displays haplotype diversity, linkage disequilibrium and differentiation that typically correspond to a small XY sex-determining region with XY males and XX females. In male alleles, traces of purifying selection were found for a *trehalose phosphatase*, an *exostosin* and a *WRKY transcription factor*, with strikingly low polymorphism levels between distant geographic regions. Both diversity and network analysis revealed that *H* alleles are more closely related to *M* than to *F* alleles.

Conclusions: Hermaphrodite alleles appear to derive from male alleles of wild grapevines, with successive recombination events allowing import of diversity from the X into the Y chromosomal region and slowing down the expansion of the region into a full heteromorphic chromosome. Our data are consistent with multiple domestication events and show traces of introgression from other Asian *Vitis* species into the cultivated grapevine gene pool.

Keywords: Dioecy, Domestication, Hermaphroditism, Sex chromosome, *Vitis vinifera* L

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Background

The wild grapevine, *Vitis vinifera* L. subsp. *sylvestris*, is the wild ancestor of the domesticated grapevine *V. v. vinifera* [1,2], cultivated for wine and table grape production [3]. The genus *Vitis*, a monophyletic taxon of the family *Vitaceae* [4,5], includes approximately sixty species present mainly in Asia and America, all of which -except the domesticated grapevine- are dioecious (male and female flowers borne on different plants) [6,7]. During grapevine domestication, flower reproductive morphology has incurred radical modifications, with the change from dioecy to hermaphroditism in domesticated grapevines [8]. The geographic origin of hermaphroditism development in the domesticated grapevine is still not elucidated, nor is it known whether it occurred during primary [1,9] and/or secondary domestication events believed to have occurred in geographically distinct areas around the Mediterranean [10,11].

Sex expression in *Vitis* flower is thought to be controlled by a major locus with three alleles, male *M*, hermaphrodite *H* and female *F*, with an $M > H > F$ allelic dominance [6,7,12-14]. Several genetic maps based on interspecific crosses have confirmed that sex determinism in the genus *Vitis* is under the control of a single major genomic region located on chromosome 2, close to the SSR marker VVIB23 [15-17]. Recently, a complex interspecific cross (*V. vinifera* x [*V. riparia* x *V. cinerea*]) was used by Fechter et al. [18] to narrow the location of the sex locus to a 143 kb genomic region located between positions 4.907.434 and 5.037.597 bp of chromosome 2 [18] on the physical map of the *V. vinifera* reference genome sequence (PN40024 12x.0 version [19]). So far, the co-localisation on chromosome 2 of the sex locus in *V. vinifera* subsp. *vinifera* has been confirmed only in the genetic map of one intra-specific cross [20], with a recombination distance of 0.4 cM from the nearest genetic marker (VVIB23). Moreover, in the *V. v. sylvestris* subspecies, the sex locus localisation remains to be confirmed.

The evolution of proper sex chromosomes is quite rare in plants: indeed, approx. 40 species of flowering plants are currently known to have developed sex chromosomes and among them, half have heteromorphic sex chromosomes [21]. A sex chromosome may start to develop in dioecious species through the suppression of recombination between male- and female-sterile mutations with complementary dominance in close proximity on a chromosome [22]. Then, this sex-determination region would gradually grow in size, increasingly incorporating sex-linked genes and eventually evolving into heteromorphic sex chromosomes [21,22]. Some of the processes involved in sex chromosome evolution, as the suppression of genetic recombination or the genetic degeneration of the Y chromosome, are not well understood and only the study of the sex-determining systems on different species and at different steps of evolution could provide some answers [23]. While the sex determination locus in *Vitis* species was mainly studied to develop genetic markers for early sexing for breeding purposes [18,20], the work of Fechter et al. [18], evidencing a small sex-determination region, suggests that *Vitis* species could be good candidates to study the early steps of sex chromosome evolution.

In the present study, we explore the sequence polymorphisms near the sex locus in a genetically and geographically diverse panel of wild and domesticated grapevines, with the objectives to: i) confirm the position and boundaries of the sex locus in *V. vinifera* subsp. *sylvestris*; ii) characterise the sex region in terms of linkage disequilibrium, genetic diversity, selection signature and candidate genes; and iii) use this information to explore the geographic and genetic origin of hermaphroditism in domesticated grapevine.

Since wild grapevines carry the ancestral form of the sex locus from which the domesticated grapevine hermaphroditism derived, we first mapped sequence polymorphisms linked to the sex trait in *Vitis vinifera* subsp. *sylvestris*. Then, we compared the polymorphisms linked to the sex trait in diverse wild and domesticated grapevine populations to study the origin of hermaphroditism in domesticated grapevines.

Methods

Plant material and phenotypic trait data

The plant material consisted of 73 wild (39 females and 34 males) and 39 hermaphrodite domesticated grapevines (Additional file 1). These grapevines were chosen among 139 wild genotypes and 2.323 domesticated genotypes [24] to maximise both genetic diversity and geographic representation. Three genotypes from other species were also considered to represent genetic variation in the subgenus *Vitis*: *V. balansaeana*, *V. coignetiae* and *V. monticola* [25]. The grapevines were sampled either in natural populations or from the French National Grapevine Germplasm Collection (INRA, Domaine de Vassal, France; http://www1.montpellier.inra.fr/vassal/). The genotypes considered varied according to the genetic analyses (Additional file 1). Sex phenotypes (male, female or hermaphrodite) were evaluated by observations of flower morphology repeated over several years, and coded according to the International Organization of Vine and Wine descriptors (code number OIV-151 [26]).

DNA extraction

DNA was extracted from 150 mg of leaves according to the Dneasy Plant Mini Kit (Qiagen) instructions with 1% Polyvinylpyrrolidone (PVP 40.000) and 1% of β-mercaptoethanol added to the buffer AP1 to eliminate polyphenols, strong inhibitors of in-vitro enzymatic reactions abundantly present in the crude grape cell lysate.

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Amplicons sequencing

Several studies located the sex locus in *Vitis* close to the SSR marker VVIB23 on chromosome 2 [15-17,20,27]. In addition, we preliminary confirmed this locus in *Vitis vinifera* subsp. *sylvestris*, using 11 SSR markers segregating in several intra-specific crosses resulting from open-pollination (data not shown). Using this information, we designed 11 amplicons to cover a region between positions 4.781.551 bp and 5.037.597 bp of chromosome 2 (PN40024 grapevine genome reference sequence, version 12×.0 [19]; Table 1, Additional file 2 for primer sequences). This region covers both the VVIB23 SSR marker and the 143 kb region as defined by Fechter et al. [18] (Figure 1). We did not extend the coverage further downstream as we found that the SSR marker VMC3b10 (position 5.057.413 bp) was not associated with sex segregation in our wild grapevine mapping populations (data not shown).

According to Fechter et al. [18], the 143 kb region of chromosome 2 (12×.0 version) between 4,907,434 and 5,050,616 bp corresponds to the female allele of the hermaphroditic Pinot Noir 40024, while the slightly different hermaphrodite allele is located on the unassigned scaffold_233 (chromosome UnRandom of the 12×.0). The 12×.0 scaffold_233 is collinear with the chromosome 2 of the 8× grape genome reference sequence [19]; both these assemblies display two regions which are absent from the chromosome 2 assembly of the 12×.0 reference sequence version: a region between the *3-Oxoacyl synthase III C terminal* (KASIII) and the *PLATZ transcriptor factor*, and the *adenine phosphoribosyl transferase* (APT3) region [18]. The APT3 distinguishes female individuals from male and hermaphroditic ones [18]. A gene, the *phosphatidic phosphatase 2* (PAP2), is not predicted by the Gaze annotation of the 12×.0 reference sequence version but it is annotated by the Gaze annotation of the 8× reference sequence version and confirmed by Fechter et al. [18] on the 12×.0 reference sequence version.

For our work, eight primer pairs out of the eleven could thus be designed using the Gaze annotation of the 12×.0 reference sequence version (Table 1, Figure 1). A primer pair (VSVV011) was developed in the PAP2 gene using the Gaze annotation of the 8× reference sequence version (Table 1). Another primer pair (VSVV010) was specifically developed to cover the region of the putative APT3 distinguishing female individuals from male and hermaphroditic ones [18]. A last amplicon (VSVV008) was designed to amplify a gene present in the region between the KASSIII and the PLATZ transcriptor on the 12×.0 scaffold_233; the predicted protein of this gene blasts with an *Ethylene Overproducer-like 1* gene (ETO1, blastx E-value = 4e-83). For the ETO1 and APT3 amplicons, the positions on the grape genome physical maps were estimated based upon a manual realignment of the unassigned scaffold_233 (chromosome UnRandom of the 12×.0) and the 8× reference sequence version respectively, on the chromosome 2 of the 12×.0 reference sequence version. As a consequence, in our work the 12×.0 positions of these two amplicons are approximate (Table 1).

All primer pairs were designed using the Primer3 software V.0.4.0 [28,29] so as to amplify stretches between 600 and 1.300 bp and cover a part of the promoter and the first exons and introns [28,29]. Thermocycling consisted of an initial stringent cycle (94°C for 3 minutes followed by 12 cycles of 94°C for 30 seconds, from 65 to 56°C decreasing by 0.70°C at each cycle for 45 seconds, 72°C for 120 seconds) and additional 25 cycles of 94°C for

Table 1 Characteristics of the amplicons used in this study to cover the sex locus and its edges

Amplicon name	Position	Amplicon size	Gene annotation*	
			Name	Putative function
VSVV001	4781551 - 4782603	1053	GSVIVT01004916001	Esterase/lipase/thioesterase family protein
VSVV002	4822617 - 4824068	1452	GSVIVT01001263001	SAUR family protein
VSVV003	4850582 - 4851997	1416	GSVIVT01001267001	Pentapeptide repeat protein
VSVV004	4861475 - 4862891	1417	GSVIVT01001269001	Yabby14 protein
VSVV005	4883461 - 4884818	1358	GSVIVT01001272001	Soluble acid invertase
VSVV006	4900275 - 4901493	1219	GSVIVT01001275001	Trehalose-6-phosphate phosphatase (TPP)
VSVV007	4921838 - 4923352	1515	GSVIVT01001277001	Exostosin family protein
VSVV008[†]	4953195 – 4954179**	984	GSVIVT01004781001	Ethylene Overproducer-like 1 (ETO1)
VSVV009	4989467 – 4990268	802	GSVIVT01001286001	WRKY transcription factor 21
VSVV010[‡]	5009549– 5010222**	673	GSVIVT00007310001	Adenine phosphoribosyltransferase (APT3)
VSVV011[§]	5036645 – 5037597	953	GSVIVT00007312001	Phosphatidic acid phosphatase 2 (PAP2)

*Gaze annotation, **Approximative values. [†]PN40024 reference sequence, 12×.0 version, amplicon position 16.072.323-16.073.307, Scaffold_233, chromosome UnRandom; [‡]PN40024 reference sequence, 8× version, amplicon position 5.192.572-5.193.382, scaffold 187, chromosome 2; [§]Primers developed in the gene predicted using the 8× Gaze annotation and confirmed by Fechter et al. [18] on the 12×.0 reference sequence version.

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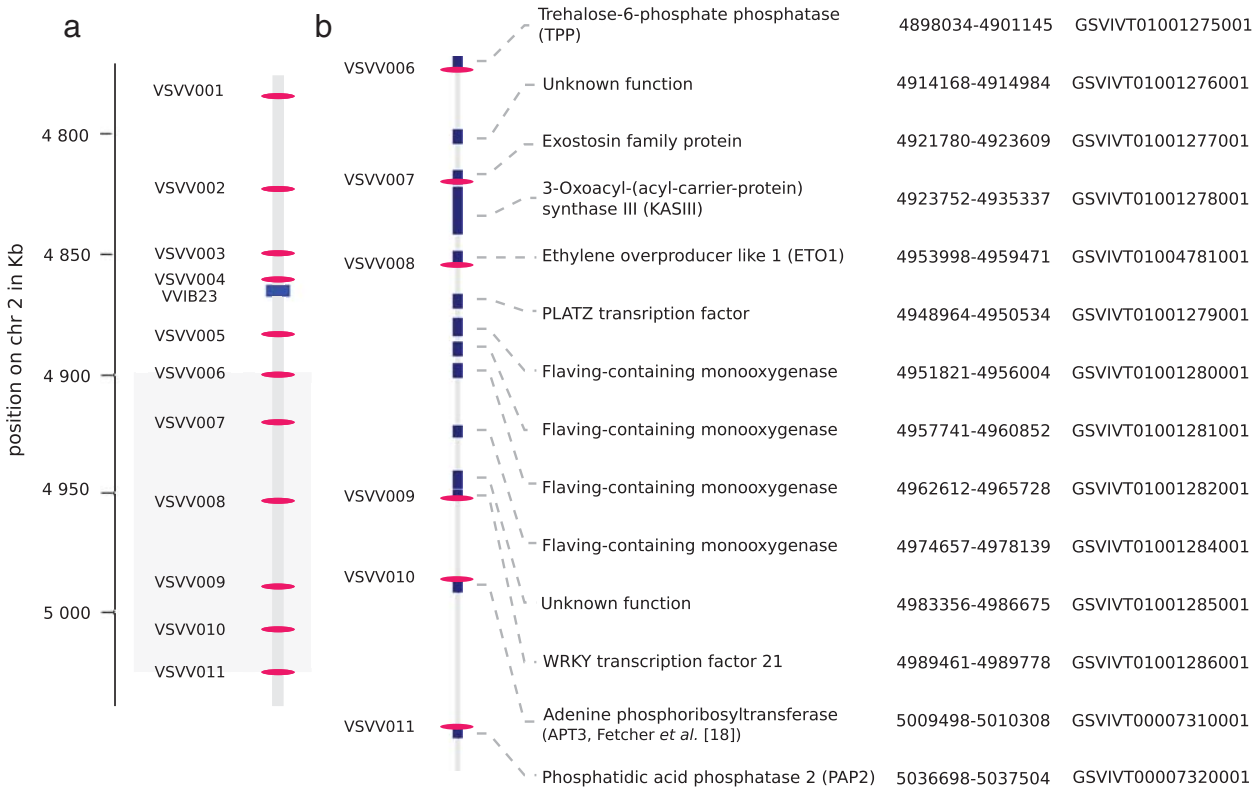

Figure 1 Amplicon position in the sex locus and its boundaries on chromosome 2 of the 12×.0 reference sequence version. a) VVIB23 SSR marker (light blue rectangle) and amplicon position (red ellipses); **b)** Amplicon position and gene Gaze annotation in the 143 kb sex locus defined by Fechter et al. [18]. The 12×.0 annotated genes version are represented in dark blue and our amplicons in red. For the APT3 and the ETO1gene, we used the synteny between the chromosome 2 of the 8X reference sequence version, the unassembled scaffold_233 of the 12×.0 reference sequence version, and the BAC sequencing maps of *V. riparia* and *V. cinerea* [18] to estimate their relative position on chromosome 2, 12×.0 version (see Methods). The phosphatidic phosphatase 2 (PAP2), is not predicted by the Gaze annotation of the 12×.0 reference sequence version but it is annotated by the Gaze annotation of the 8× reference sequence version and confirmed by Fechter et al. [18] on the 12×.0 version.

30 seconds, 56°C for 45 seconds, 72°C for 90–120 seconds. Sequencing was performed on PCR products purified using the AMPure® kit (Agencourt®, MA, USA); BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied BioSistem, CA, USA) was used following the standard protocol and reaction products were purified with the CleanSEQ® kit (Agencourt) and read on a 3130×l Genetic Analyzer (Applied BioSystems). Raw sequence files (AB1 format) were imported, aligned and trimmed using the Staden software v.2.0.0 [30]; SNP calling was carried out manually using the Staden interface. Then, fasta files were exported and subsequently analysed in other softwares and pipelines.

Identification of sequence polymorphisms linked to the sex trait

Phenotypic sex inheritance in wild grapevines produces only male and female variants, with a ratio near to 1:1 in adult populations (even if some variation in sex phenotypes have been observed [13,26], in our sample only two morphs were found, M and F). The most parsimonious hypothesis we could make on sex inheritance in grape, based on previous observations, preliminary data analysis, and literature survey [6,7,17,18,20], was that of a XY system, where, at the sex locus, the female is homozygous (XX) and the male is heterozygous (XY).

To map the sex locus on the genome, we first used a genetic association approach, looking for correlations between sex flower phenotypes and sequence polymorphisms in a panel of diverse wild genotypes from different geographic provenances (Additional file 1). However, the use of general or mixed linear models searching for association resulted in too many false positives (SNP that were correlated to sex but explained only a portion of the phenotypes). Thus, we used an approach similar to Siegismund [31], using Fisher tests to compare, for each polymorphism and for male and female wild grapevines separately, the expected and observed proportions of homozygous and heterozygous genotypes. The expected proportions were assumed to follow the Hardy–Weinberg law and were calculated from the allele frequencies observed in the entire population (sum of male and female individuals). The observed counts were the number of homozygous and heterozygous genotypes

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actually recorded in male and female grapevines. Indels were coded as present/absent (Additional file 3). Fisher tests were calculated with the *fisher.test* function of the R statistical software [32]. We only considered sequence polymorphisms with less than 20% missing data and with a minimum allele frequency in the sample higher than 5%. A test was considered significant when the probability of deviation from the null hypothesis was inferior to a 0.05 P-value threshold adjusted by a Bonferroni correction for multiple hypotheses testing (0.05/n with n corresponding to the total number of studied polymorphisms).

Linkage disequilibrium in the sex region

To explore linkage disequilibrium between and within amplicons covering the sex region, we used the *Measure.R2VS*() function in the R package LDcorSV [33]. r^2VS is the square of each pairwise correlation corrected by both the relatedness and genetic structure of the sample [33]. The sample considered here was composed of 18 male and 18 female individuals (Additional file 1). These 36 specimens were chosen among those with the least missing data, eliminating the most closely related individuals and equilibrating their geographic representation. The genetic structure matrix was calculated from a dataset of 20 SSRs [24] of all the wild genotypes in this study, using STRUCTURE software [34]. We used the model with uncorrelated allele frequencies, admixture, and no prior population information, previously showed to be pertinent in grapevine [35]. Ten STRUCTURE runs (each with 5×10^5 iterations and 5×10^5 replicates) for each K-level were obtained and compared to estimate group assignation stability. The most probable number of sub-populations was inferred based on both the similarity pattern among the 10 STRUCTURE replicates and Evanno's Δks statistics [36]. The kinship matrix was obtained using ML-Relate software [37] with the same SSR markers and genotypes as above.

Diversity in *M*, *F* and *H* haplotypes and signature of selection

To compare the diversity of male, female and hermaphrodite alleles at the significant sex-linked amplicons (see Additional file 1 for the genotypes considered), the haplotypes were reconstructed using PHASE v2.1 with default parameter values [38,39]. The attribution of individual haplotypes to the *M*, *F* and *H* groups (called hereafter haplogroups) were carried out with the help of haplotype trees (Additional file 4) built with a maximum likelihood method (PhyML 3.0 [40]) implemented in SeaView v4.3.3 [41] and based on the Generalised Time-Reversible (GTR) model [42].

Genetic diversity in *M*, *F* and *H* haplotypes was evaluated with the following statistics: number of haplotypes (Nh), number of segregating sites (S), haplotype diversity (H) and nucleotide diversity (π). In order to detect a signature of selection in the sex region, Tajima's D [43] and Fu and Li's D* [44] statistics were calculated with the DnaSP v5 software [45] separately for the male, female and hermaphrodite haplogroups. To confirm traces of selection detected on the male haplogroups with the Tajima's D and the Fu and Li's D* tests, the E statistics and the DH test [46] were computed using the male haplotype of *V. balanseana* as an outgroup (Table 2).

Finally, we evaluated the intraspecific genetic differentiation between male, female and hermaphrodite haplogroups, and the interspecific differentiation between *V. v sylvestris* and *Vitis* species haplotypes, using the Fst statistics [47,48] with DnaSP v5 software as well. The *Vitis* species used for this statistics were *V. balanseana*, *V. monticola* and *V. coignetiae*.

Origin of the H haplotypes

Combining the haplotypes of the four sex-linked amplicons, the *M*, *F* and *H* macrohaplotypes were reconstructed. PHASE v2.1 was run again using a 100 burn-in period with 100 iterations with a thinning interval of 1 and 10 repeats. The algorithm was run several times, validating convergence. Then, to understand the origin of *H* haplotypes in the domesticated grapevine, a network analysis was carried out on the *F*, *M* and *H* macrohaplotypes using the median-joining method as described in Bandelt et al. [49] and implemented in Network v4.6.1.1. [50]. A Star Contraction was run before running the network calculation.

Finally, the relationship between the network distances (in number of mutations) of the *H* haplotypes from the *M* haplogroup, and the geographic origin, grape use (table, wine or both), degree of domestication (ancient

Table 2 Allocation of 0, 1 or 2 female haplotypes (*F*) to the hermaphrodite, male and female genotypes, according to the maximum likelihood trees, for the four sex linked amplicons

Genotype	VSVV006	VSVV007	VSVV009	VSVV010
Hermaphrodite	*n = 22*	*n = 22*	*n = 22*	*n = 21*
0 haplotype *F*	1	0	1	9
1 haplotype *F*	21	22	19	8
2 haplotypes *F*	0	0	2	4
Male	*n = 22*	*n = 22*	*n = 22*	*n = 18*
0 haplotype *F*	0	0	0	0
1 haplotype *F*	22	22	22	18
2 haplotypes *F*	0	0	0	0
Female	*n = 24*	*n = 24*	*n = 24*	*n = 22*
0 haplotype *F*	0	0	0	0
1 haplotype *F*	0	0	0	0
2 haplotypes *F*	24	24	24	22

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or modern cultivars [51]) and the genetic structure ancestry of the domesticated grapevines [35] were explored using an ANOVA.

Results

Sequence polymorphisms linked to the sex trait

Eleven amplicons representing 9.523 bp in total and designed to partly amplify gene sequences were chosen to cover the sex locus and its boundaries [18,20]. Sequencing these 11 amplicons on a sample of 65 genetically and geographically diverse wild genotypes (31 males and 34 females, Additional file 1, [GenBank: KJ575622-KJ57662]), allowed the identification of 146 polymorphic sites (Additional file 3): 137 SNPs and 9 indels. Thirty-six SNPs were located in introns and twenty in exons, among which ten were non-synonymous. The allele frequencies of 51 and 64 polymorphisms in female and male genotypes respectively were found significantly deviating from the Hardy–Weinberg proportions (Figure 2b). These significant polymorphisms were mainly found in VSVV006, VSVV007, VSVV009 and VSVV010 (87,04% of the significant polymorphisms in females and 90.60% in males).

Among the significant polymorphisms, 28 perfectly fitted the XY sex determination model. For these polymorphisms, 100% of the male genotypes were heterozygous and 100% of the female genotypes were homozygous for the most frequent allele, i.e. for example males were A/T and females were A/A but never T/T (Figure 2c, Additional file 3). In hermaphrodite domesticated genotypes, these same polymorphisms were in the majority of cases in a heterozygous state (Additional file 5). These 28 polymorphisms, perfectly fitting the XY model, were only found in the VSVV006, VSVV007 and VSVV009 amplicons and 3 of them resulted in non-synonymous amino acid changes (38th, 61th and 66th polymorphism in VSVV006 or VSVV007, Additional file 3).

Moreover, 18 significant polymorphisms in VSVV006, VSVV007, VSVV009 and VSVV010 were only slightly deviating from the XY sex determination model, with all female genotypes homozygous for the most frequent allele and one or two non-heterozygous males (Additional file 3). For example, for the polymorphism 126 (crosses in Figure 2b, c) corresponding to the sex-linked indel in the second intron of the APT3 gene [18], all female were homozygous without the indel while 92% of male were

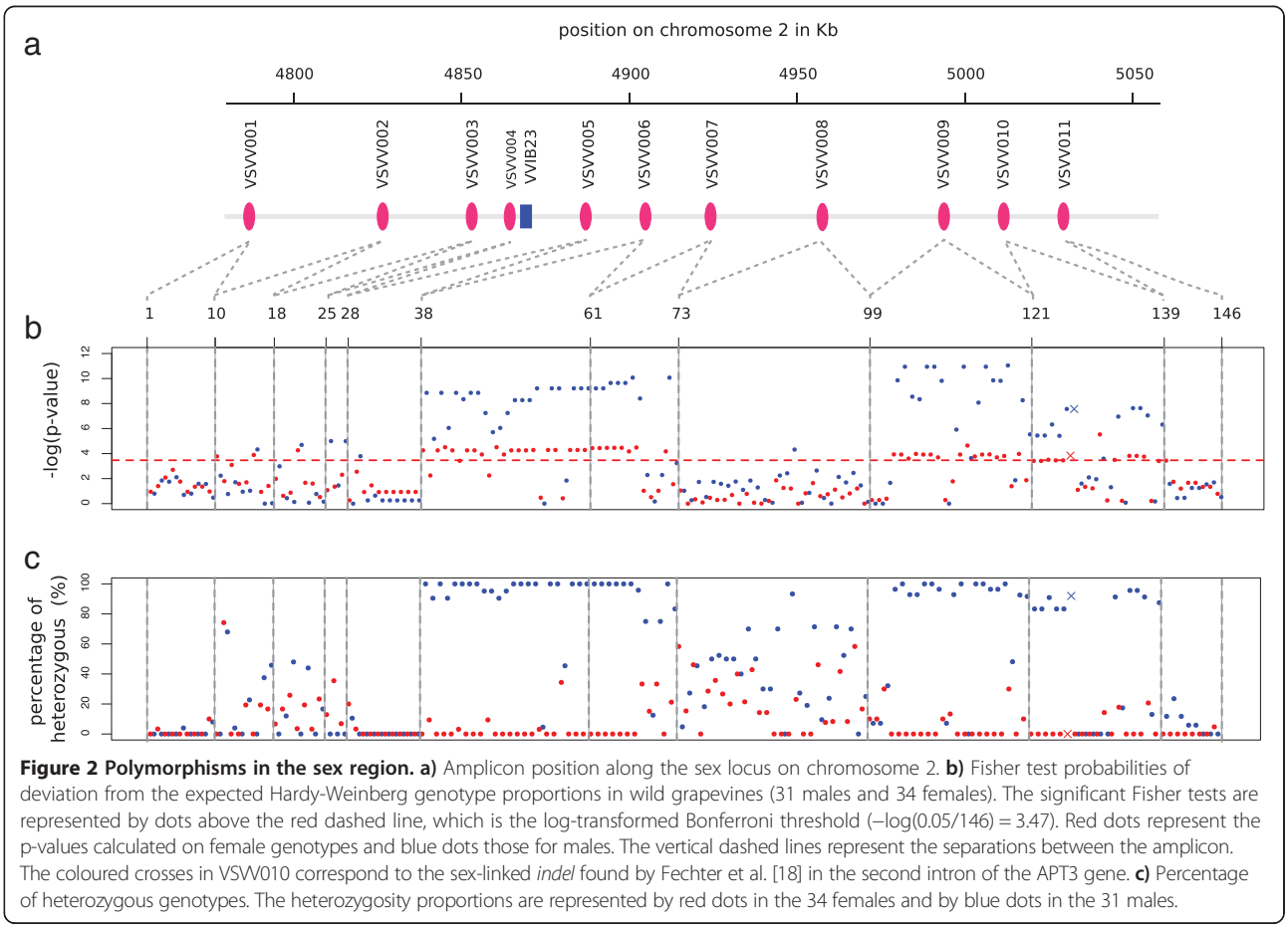

Figure 2 Polymorphisms in the sex region. a) Amplicon position along the sex locus on chromosome 2. **b)** Fisher test probabilities of deviation from the expected Hardy-Weinberg genotype proportions in wild grapevines (31 males and 34 females). The significant Fisher tests are represented by dots above the red dashed line, which is the log-transformed Bonferroni threshold (–log(0.05/146) = 3.47). Red dots represent the p-values calculated on female genotypes and blue dots those for males. The vertical dashed lines represent the separations between the amplicon. The coloured crosses in VSVV010 correspond to the sex-linked *indel* found by Fechter et al. [18] in the second intron of the APT3 gene. **c)** Percentage of heterozygous genotypes. The heterozygosity proportions are represented by red dots in the 34 females and by blue dots in the 31 males.

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heterozygous (23 heterozygous, one homozygous with the indel and one homozygous without it) (Additional file 3). In the VSVV008 amplicon, only one SNP was found slightly deviating from the XY sex determination model (Figure 2b and Additional file 3).

By contrast, and although few of them were found significantly departing from Hardy-Weinberg proportions (Fisher test), the polymorphisms found in VSVV002, VSVV003, VSVV004 and VSVV005, largely deviated from the XY model, particularly in male genotypes (Figure 2 and Additional file 3).

In summary, 46 significant polymorphisms in the VSVV006, VSVV007, VSVV009 and VSVV010 amplicons fitted either strictly (28) or closely (18) the XY sex-determination model. These results allowed us to define the boundaries of the sex locus at the positions 4.884.818 and 5.036.645 on chromosome 2 of the PN40024 physical map (12×.0 version). This 151,83 kb region, externally delimited by the gene fragments VSVV005 and VSVV011 contains 13 candidate genes (Figure 1 and Additional file 6).

Linkage disequilibrium in the sex region

The intra- and inter-amplicon linkage disequilibrium (LD) was estimated on a sub-sample of 18 male and 18 female wild grapevines (Additional file 1), by calculating the pairwise square correlation coefficient r^2VS [33], correcting for the structure and kinship of the sample. Only sequence polymorphisms with less than 20% missing data and with a 0.2 minor allele frequency were analysed. At these thresholds, no polymorphisms were retained in the VSVV001 fragment.

The highest values of LD were found within and between the four sex-linked fragments (Figure 3). The mean LD for all pairwise comparisons for the four sex-linked fragment was $r^2VS = 0.72$ for a total physical length of 109.76 kb. The maximum mean intra-amplicon LD was $r^2VS = 0.84$ over 374 bp for VSVV010 and the minimum was $r^2VS = 0.63$ over 504 bp for VSVV009. The maximum inter-amplicon LD was $r^2VS = 0.81$ between VSVV006 and VSVV010 (109.39 kb) and the minimum was $r^2VS = 0.63$ in between VSVV007 and VSVV009 (67.84 kb). The fragment VSVV008 (only weakly linked to sex) presented a significant but lower LD with the sex-linked fragment ($r^2VS = 0.31$).

Diversity of the *M*, *F* and *H* haplotypes and signature of selection

The *M*, *F* and *H* haplotypes for the four sex-associated amplicons (VSVV006, VSVV007, VSVV009 and VSVV010) were assigned using maximum likelihood haplotype trees. According to the XY model and the rules of dominance described for *Vitis* ($M > H > F$ [6,7,12-14]), the haplogroup containing haplotypes from female, male and hermaphrodite genotypes was designated as the female *F* haplogroup (Additional file 4); it is supposed to contain the *F*

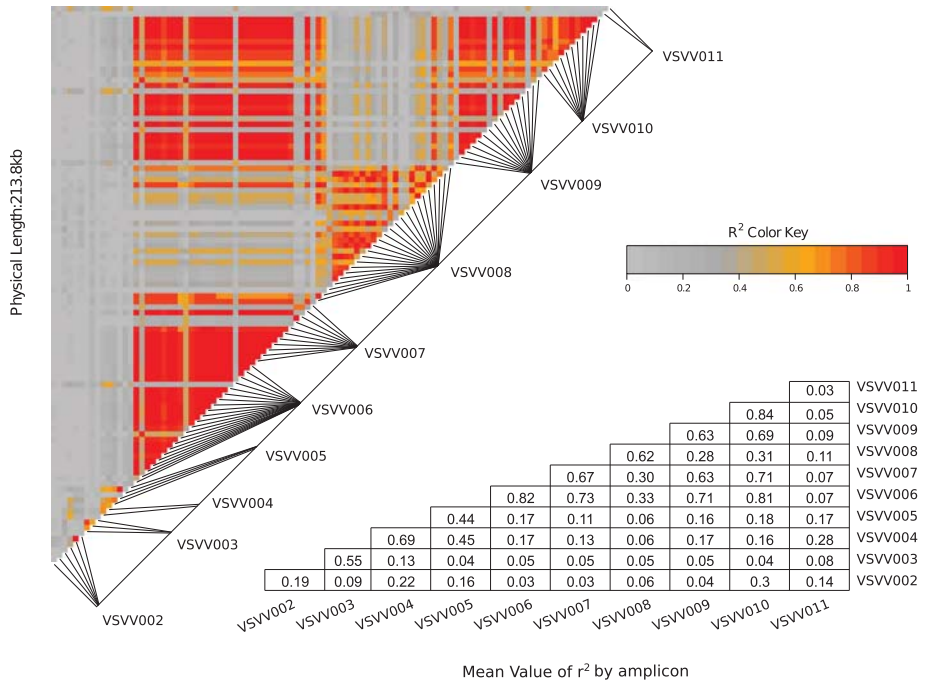

Figure 3 Linkage disequilibrium plot based on r^2_{VS} values for the SNPs and indels of the sequenced amplicons. Only polymorphisms with a major allele frequency > 0.2 were used (none were retained in VSVV001 because of this filter). Indels were coded as present/absent. Bottom table: average LD estimates within amplicon and between amplicon pairs.

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haplotypes of *FF* females, *MF* males and *HF* hermaphrodites genotypes. By difference, the alternate haplotypes found in male and hermaphrodite genotypes but not present in the *F* haplogroup, were considered as the *M* and the *H* haplotypes respectively (Additional file 4).

For the wild female and male genotypes, the number of *F* haplotypes found in the female haplotype group trees was consistent with the XY sex model (one *F* haplotype in male genotypes and two in females; Table 3). However, some hermaphrodite genotypes presented, for one or two amplicons only (never for the four amplicons simultaneously) either no or two *F* haplotypes. This departure from the sex model was particularly pronounced in VSVV010.

For diversity parameters calculation and the estimation of selection signature, we differentiated the *F* haplotypes of the hermaphrodite domesticated genotypes from the

Table 3 Diversity statistics for wild male/female, cultivated hermaphrodite and female haplotypes groups

	VSVV006	**VSVV007**	**VSVV009**	**VSVV010**
	1111 bp	***849 bp***	***690 bp***	***498 bp***
Wild male haplotypes				
Effective	22	22	22	18
S	5	1	6	12
Nh	3	2	3	6
H	0.18	0.09	0.18	0.72
π	0.00041	0.00011	0.00079	0.00375
Tajima's D	−1.99 *	−1.16 (ns)	−2.07 *	−1.71 (ns)
Fu and Li's D*	−2.91*	−1.57 (ns)	−3.23 **	−2.10 +
Zeng et al.'s E	−1.404*	−0.866 (ns)	−0.551(ns)	−0.334 (ns)
DH test (p-value)	0.148 (ns)	0.331 (ns)	0.023 **	0.035**
Domesticated hermaphrodite haplotypes				
Effective	22	22	20	26
S	11	2	3	11
Nh	6	3	4	5
H	0.72	0.26	0.36	0.46
π	0.00216	0.00031	0.00091	0.00474
Tajima's D	−0.71 (ns)	−1.18 (ns)	−0.69 (ns)	−0.60 (ns)
Fu and Li's D*	0.53 (ns)	−0.63 (ns)	−0.12 (ns)	0 (ns)
Wild female haplotypes				
Effective	71	71	71	62
S	13	6	26	19
Nh	12	7	16	17
H	0.69	0.47	0.86	0.72
π	0.00136	0.00170	0.00526	0.00744
Tajima's D	−1.24 (ns)	0.38 (ns)	−1.01 (ns)	−0.26 (ns)
Fu and Li's D*	−1.81 (ns)	0.24 (ns)	0.15 (ns)	1.28 (ns)
Domesticated female haplotypes				
Effective	21	22	20	16
S	7	4	9	16
Nh	6	3	8	9
H	0.77	0.26	0.77	0.86
π	0.00224	0.00090	0.00500	0.01089
Tajima's D	0.89 (ns)	−0.85 (ns)	1.24 (ns)	0.49 (ns)
Fu and Li's D*	0.66 (ns)	1.10 (ns)	0.86 (ns)	0.93 (ns)

S = number of segregating sites, Nh = number of different haplotypes, H = haplotype diversity and π = nucleotide diversity. For the Tajima's D values, Fu and Li's D*, Zeng et al.'s E and DH test : "**" indicate a p-value < 0.01, "*" a p-value < 0.05, "+" a p-value < 0.10 and (ns) non-significance. The E statistics and the DH test were computed using the male haplotype of *V. balanseana* as an outgroup.

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F haplotypes of the male and the female wild genotypes, so as to detect different diversity or selection patterns between the domesticated and the wild compartments. Except for VSVV010, *M* haplogroups presented the lowest number of haplotypes (Nh), and the lowest level of haplotype (H) and nucleotide (π) diversity, revealing the predominant occurrence of one major haplotype, with a low number of SNPs in rare variants (Table 2). The extreme case was the VSVV007 amplicon for which only two haplotypes were observed, differing by only one SNP over 849 bp (polymorphisms n. 3 in Figure 4). On the other hand, in VSVV010, the *M* haplogroups revealed a high haplotype diversity

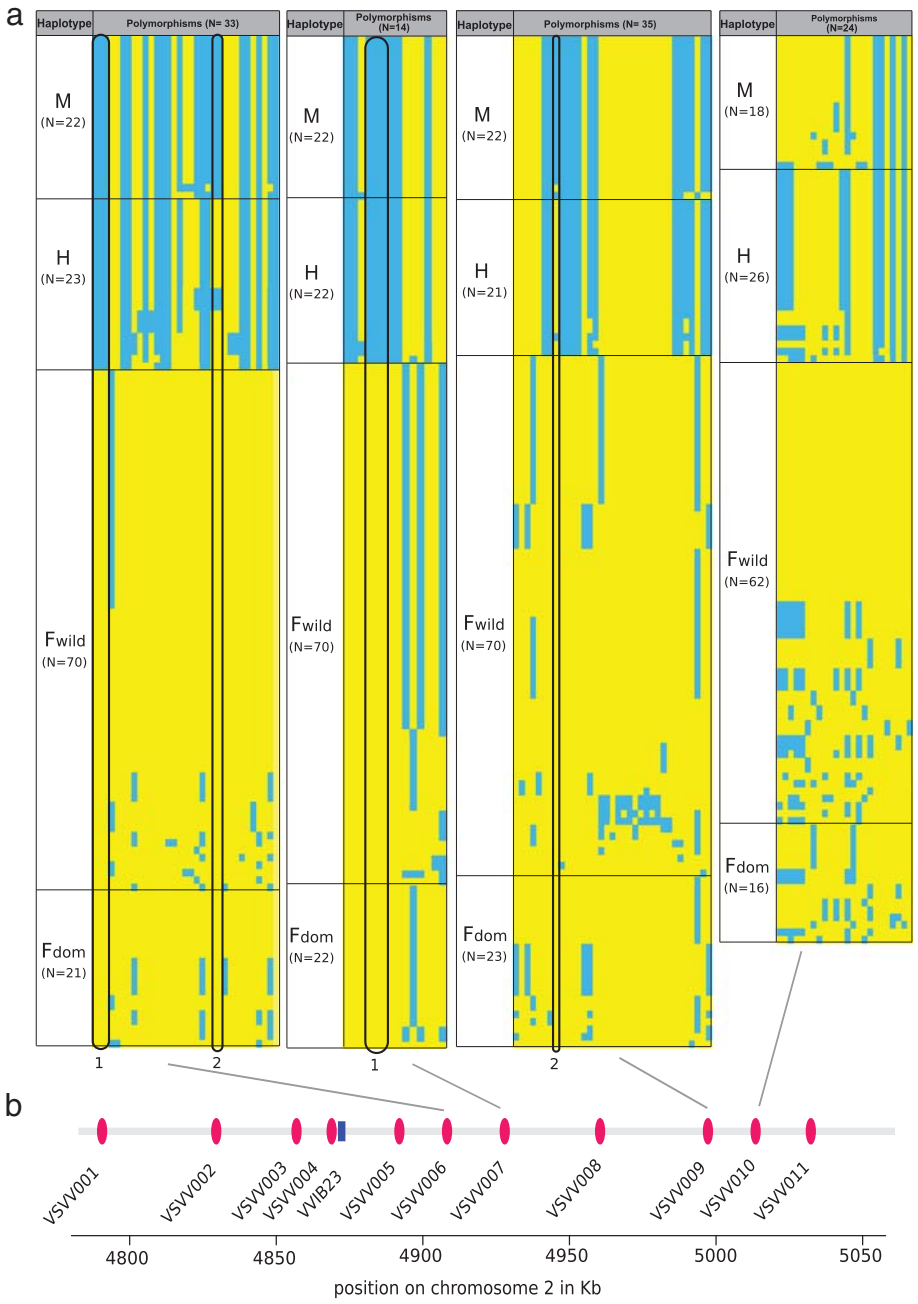

Figure 4 Sex haplotypes found in the four sex-linked amplicons. a) Haplotype details by sex : *M* = males, *H* = hermaphrodites, *F wild* = female haplotypes found in wild grapevine, and *F dom* = female haplotypes found in domesticated grapevines. Columns represent the segregating sites in the sex-linked amplicons, with the major allele in yellow and the minor allele in blue. The polymorphisms headed with the number 1 (in black) allow discriminating *F* haplotypes from *H* and *M* haplotypes; those headed with 2 allow differentiating *M* haplotypes from the *H* and *F* haplotypes. **b)** amplicon position on the sex locus on the grapevine chromosome 2.

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equivalent to the domesticated and wild *F* haplogroups, and a higher π value than for other amplicons (Table 2). The *F* haplogroups of the wild and domesticated genotypes presented strikingly more numerous and diverse haplotypes than the *M* haplogroups. Overall, domesticated and wild *F* haplogroups presented similar diversity patterns.

The *H* haplogroups showed an intermediate diversity pattern between the *M* and *F* haplogroups, but closer to the *M* haplogroups (Table 2). For VSVV010, the *H* haplogroup presented diversity patterns quite equivalent to that of *M* haplogroups, except for a lower haplotype diversity.

To illustrate these findings, the haplotypes identified for each sex-linked amplicon are presented in Figure 4 (for genotype and geographic details see Additional file 7).

This dataset shows that the three grapevine flower sexes, male, female and hermaphrodite, could be correctly predicted in 97% of the genotypes of our geographically representative *V. vinifera* sample, using a few SNPs, i.e. n. 4 to 7 of VSVV007 and n. 8 of VSVV010 (identified by black circles respectively termed 1 and 2 in Figure 4a).

Male haplogroups revealed significantly negative Tajima's D, and Fu & Li's D* values for VSVV006 and VSVV009 (Table 2). For VSVV010, the Fu and Li's D* statistics were close to the significant threshold ($0.10 > p\text{-value} > 0.05$). For male haplogroups (Table 2), all amplicon revealed negative E values, but only VSVV006 showed a significant excess of low-frequency variants. The DH tests detected significantly positive selection on VSVV009 and VSVV010. No other sex haplogroup showed significant signature of selection.

The Fst values (Table 4) revealed a wide genetic distance between the *M* and *F* haplogroups for the four sex-linked amplicons, though less pronounced for VSVV010. The *H* haplogroups were genetically closer to *M* than to *F* haplogroups. For VSVV007, the *H* and *M* haplogroups bore identical haplotypes, thus displaying a null distance. Comparatively, slight genetic differences only were found between the wild and the domesticated *F* haplogroups in VSVV006, VSVV009 and particularly VSVV010. However, for VSVV007, the wild and the domesticated populations of *F* haplogroups seem to be distinct. All genetic differentiation values were lower in VSVV010, revealing that all sex haplogroups are less differentiated in this region. For the four amplicons, the intra-specific genetic distances between male (or hermaphrodites) and female haplogroups were largely superior to the interspecific genetic distance between *Vitis* sp. haplotypes (Table 4).

Origin of the H allele

To determine the origin of the *H* allele, a network was built based on *F*, *M* and *H* macrohaplotypes, combining information provided by the four sex-linked amplicons (Figure 5a). According to this haplotype network, where the distance between pairs of genotypes is proportional to the number of mutations between them, *H* macrohaplotypes were closer to the *M* ones than to the *F* macrohaplotypes. The network displayed two groups of *H* macrohaplotypes: the first (*H1*), at the edge of the network, was only composed of three domesticated grapevines: *cv. Tsolikouri (chTSO), cv. Ak ouzioum Tapapskii (chAKO)* and *cv. Sylvaner (chfSYLVA)*, while the second, *H2* grouped all the others *H* macrohaplotypes of the domesticated hermaphrodite grapevines. The *M* macrohaplotypes of the wild male grapevines were located between the two *H* macrohaplotypes groups. However, one male wild macrohaplotype, *Lambrusque Ul any nad Zitavou A07 (smUNZA07)* from Slovakia, displayed a macrohaplotype closer to the *H2* macrohaplotypes than to the other *M* macrohaplotypes (Figure 5a). This grapevine displayed a VSVV007 haplotype not found in other wild male grapevines, but found in two domesticated hermaphrodite genotypes. Concerning the *F* macrohaplotypes, 3 subgroups could be defined according to the occurrence of wild or domesticated macrohaplotypes (Figure 5a,b). The *F1* group was composed by a majority of wild macrohaplotypes together with 4 cultivars: *cv. Cabernet franc (chCAF), cv. Sylvaner (chfSYLVA), cv. Lignan (chfLN)* and *cv. Lameiro (chLAR)*. The *F2* group contained mostly domesticated macrohaplotypes. In this group, some domesticated grapevines had two identical haplotypes allocated to the *H* haplogroup in the VSVV010; the macrohaplotypes closest to the *F1* and *F3* groups are *cv. Dattier noir (chDTN), cv. Muscat à petits grains blanc*

Table 4 Fst values between combinations of the four sex haplotype groups

Haplotype groups	Fst			
	VSVV006	VSVV007	VSVV009	VSVV010
Vitis vinifera intraspecific comparaison				
Wild males vs. wild females	0.95	0.93	0.88	0.62
Wild males vs. domesticated hermaphrodites	0.62	0.00	0.61	0.54
Domesticated hermaphrodites vs. wild females	0.90	0.92	0.86	0.67
Wild females vs. domesticated females	0.17	0.62	0.16	0.08
Vitis sp. vs *Vitis vinifera sylvestris*	0.16	0.04	0.05	0.19

The *Vitis* species used for the interspecific statistics were *V. balanseana*, *V. monticola* and *V. coignetiae*.

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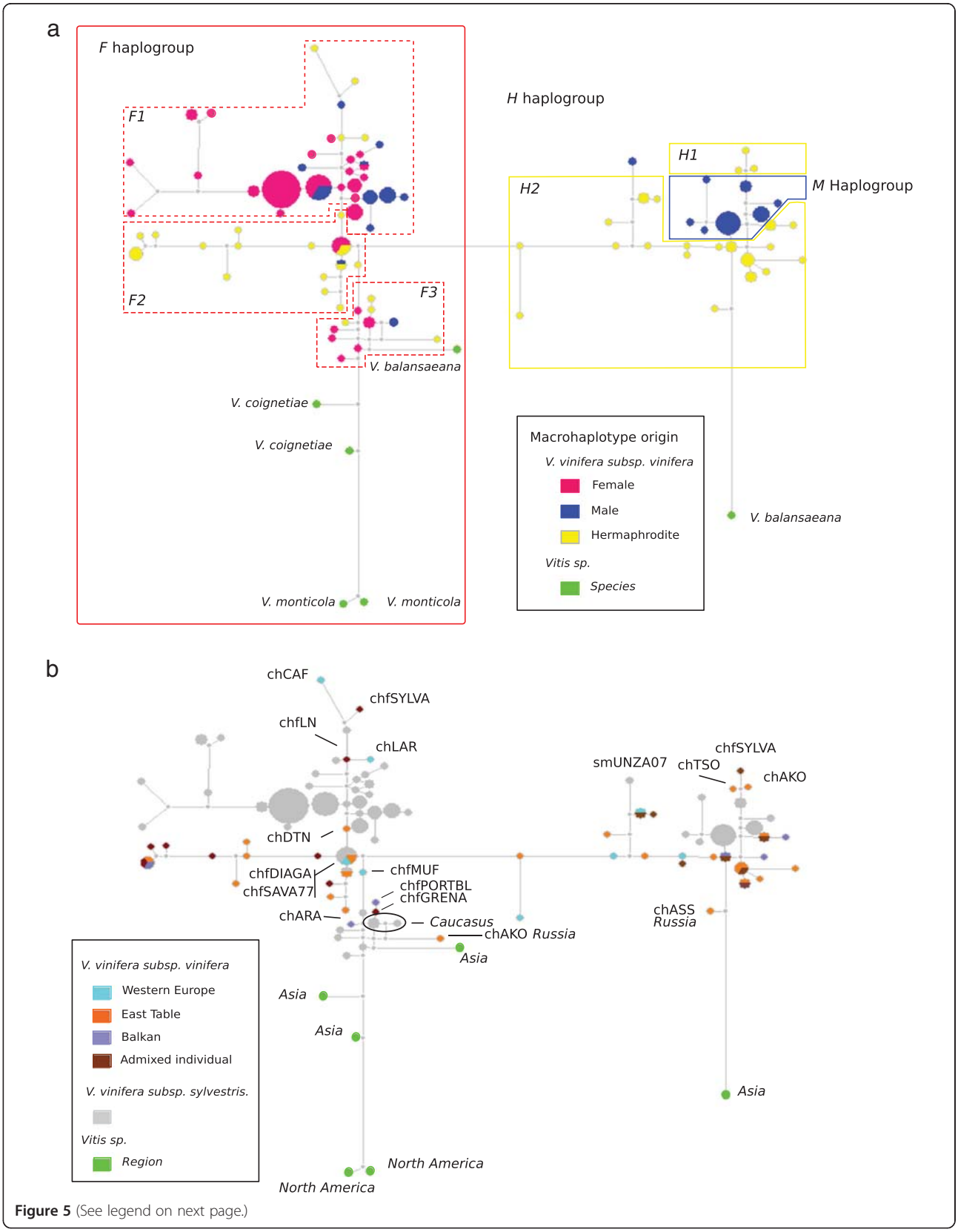

Figure 5 (See legend on next page.)

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(See figure on previous page.)
Figure 5 Consensus network carried out on the *F*, *M* and *H* macrohaplotypes. Coloured circles regroup together identical haplotypes, with size proportional to their numbers. The distance between pairs of genotypes is proportional to the number of mutations between them. **a)** Pie colours indicate the proportion of phenotypic sex morphs within the group (see legend). Polygons regroup the sex macrohaplotypes; for example, the F haplogroup regroups the 2 female macrohaplotypes of the female genotypes plus the single F macrohaplotype of the males and of the hermaphrodites. **b)** Pie colours indicate the STRUCTURE group of Bacilieri et al. [35]. The shortened name of some hermaphrodite domesticated grapevines are indicated (Additional file 1) as an example.

(chMUF), *cv. Diagalves (chfDIAGA)* and *cv. Savagnin (chfSAVA77)*. A last group *F3* grouped in approximately the same proportions wild and domesticated macrohaplotypes, among which *cv. Portugais bleu (chfPORTBL)*, *cv. Grenache (chfGRENA)*, *cv. Ak ouzioum tpapskii (chAKO)* and *cv. Araklinos (chfARA)*.

To better understand the origin of the *H* alleles, we explored the relationship between the network distances of the *H* macrohaplotypes from the *M* ones (Figure 5b) and the geographic origin, use and degree of domestication [51] of the cultivated grapevines. None of these characteristics revealed a clear correlation with *H* macrohaplotypes positions in the phylogenic network. We then tried to match the network distances with the STRUCTURE groups defined in Bacilieri et al. [35]. This work, based on 2.096 domesticated genotypes, has revealed four main genetic groups: a) wine cultivars from Western regions, b) table grape cultivars from Eastern Mediterranean, Caucasus, Middle and Far East countries, c) wine cultivars from the Balkans and East Europe, and d) a large group of cultivars with admixed genomes. Here, ANOVA analysis revealed a weak tendency ($r^2 = 0.15$, $p = 0.09$) for the Balkan and East Europe cultivars macrohaplotypes, as compared to wine Western cultivars, to be closer to the wild M macrohaplotypes.

Similarly, although pointing to different "degrees of domestication", the 3 groups of F macrohaplotypes defined above did not show a clear geographic or genetic structure pattern that could explain group composition.

The network position of the macrohaplotypes of the two female *V. monticola*, *V. coignetiae* and the male *V. balansaeana* grapevines used as outgroups, were distributed coherently to their sex phenotype: both macrohaplotypes in the *F* macrohaplogroups for the females, one in the *F* macrohaplogroups and one close to the *M* and *H* macrohaplogroup ones for the male. The closest domesticated macrohaplotypes to the *V. balansaeana* ones belonged to two Russian cultivars : *cv. Assyl kara (chASS)* and *cv. Ak ouzioum tpapskii (chAKO)* (Figure 5).

Discussion

Sex region location in *Vitis vinifera* subsp. *sylvestris*

From a locus defined by previous works on inter-specific crosses [17,18], 11 genes were partially sequenced on a diverse set of male and female wild grapevines. Forty-six polymorphisms in four amplicons were found perfectly or strongly linked to flower sex, allowing to locate in *V. v.* subsp. *sylvestris* a sex locus of 151.8 kb on chromosome 2, in full agreement with the 143 kb sex region defined by Fechter et al. [18] on a *Vitis* interspecific cross. Our results corroborates the dominance of the *M* allele over the *F* allele, characteristic of a XY sex-determination model, coherently with sex segregations in controlled crosses [7,12,14]. We also confirmed that the sex locus is situated downstream of SSR marker VVIB23, while previous studies, based on a lower marker density, had placed it upstream [17,20].

Within the 151.8 kb sex region, the polymorphisms of the centrally located VSVV008 amplicon associated only weakly with the sex trait, with one significant SNP only and no perfect M/F association. One hypothesis to explain this pattern may be that local recombination disrupted the association pattern in *V. v. sylvestris*. Unfortunately, in our work we were not able to unequivocally confirm the VSVV008 position within the sex locus. Actually, the PCR primers for this amplicon were designed based on the synteny between several genome sequence assemblies: the chromosome 2 of the 8× grape genome, the putative hermaphrodite allele on the unassigned scaffold_233 (12x.0) and the male *V. cinerea* BAC sequencing map [18], where VSVV008 is located as expected between VSVV007 and VSVV009. According to this information, we expected that VSVV008 would amplify only in males; however, in our *V. sylvestris* sample, it amplified indifferently of sex. Even if the sequence obtained or its PCR primers did not blast anywhere else in the genome some doubts still remain about the true coordinates of VSVV008; only new specifically designed experiments may help to definitely confirm the VSVV008 position.

Characterisation of the sex locus

Over the four genes linked to sex, we found a strong LD, unprecedented in *Vitis vinifera*, with a mean r^2VS of 0.72 over 109.76 kb. In *V. vinifera*, LD has been shown to decay rapidly: in more than 200 gene fragments, Lijavetsky et al. [52] observed an LD decay lower than 0.2 at around 200 bp, a finding later confirmed through massive genotyping by Myles et al. [53]. A larger LD in the sex locus, as compared to other genome regions, could be an indication of suppression of recombination, a feature typical of heteromorphic XY-like chromosomal regions [23].

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The lowest values of haplotype diversity (H) were found in male haplotypes of wild grape, with the predominant occurrence of one major haplotype, distributed without variation over largely diverse geographic origins, from Eastern to Western European, Caucasian and North African provenances. Hermaphrodite domesticated grapes displayed haplotype diversity values higher than that in wild males, while female haplotypes had the highest values, without notable differences between wild and domesticated pools. The large Fst values between males and females confirm the clear genetic differentiation between the *M* and the *F* haplotypes. The negative significant Tajima's D and the Fu and Li's D* values in *M* haplotypes of VSVV006 and VSVV009 indicate an excess of rare polymorphisms, revealing purifying selection. Using *V. balanseana* as outgroup, the Zeng et al. E statistics and DH test [52] confirmed this pattern for VSVV009. For the VSVV007 *M* haplotypes, these statistics were negative but not significant, probably because over its 849 bp length, we found only one segregating site. Such monomorphism may be a signal of stabilising selection, in particular because our grape samples originated from very diverse geographic regions. Indeed, in grapevine, previous works evidenced a much higher variation rate, with an average of 1 SNP in 47–129 base pairs, according to the genome region and the population studied [52,54,55]. By contrast, the *F* haplotypes for the four sex-linked presented no significant traces of selection, suggesting that these alleles are evolving under a neutral model.

Overall, the sex region presents traits typical of a small XY non-recombining region [21]. According to the commonly accepted model of sex chromosome evolution in plants, such a region can appear in dioecious species when recombination suppression occurs between two closely located male- and female-sterile mutations [22]. The *F* allele is expected to contain a recessive, "loss-of-function" type, male sterility mutation whereas the *M* allele would harbour a fully-functioning male fertility allele with, at a nearby locus, a dominant female sterility mutation [23]. In such a case, the *M* allele is expected to be constrained by selection against a recombination between the two sex-determining loci, since recombination may bring either total sterility, or reversion to the ancestral hermaphrodite state. The accumulation of insertions, inversions, repeated elements and chromosomal rearrangements in the X and the Y counterparts [56] may add to this mechanism, impeding local chromosome pairing at meiosis. Indeed, in this locus, Fechter et al. [18] reported the presence of additional repeated FMO elements and of a retrotransposon in the female allele, both absent from the male allele. These structural differences may help repress local recombination between M and F alleles. The suppression of the recombination may in turn be at the origin of the linkage disequilibrium, and it may as well explain part of the reduction of diversity in *M* alleles.

In this region, the weaker association with the sex trait in a distal (VSVV010) and, if accurately located, a central (VSVV008) genes could be a trace of some recombination events, sufficient to break the association with the sex causal genes, but not ample enough to completely blur LD traces (Figures 2 and 3). Rare recombination events could have prevented the evolution of this small sex region into a full sex chromosome in *Vitis*, although dioecy is supposed to have appeared in this taxon millions of years ago [57]. Finally, if the VSVV008 is well located in the sex locus, sex determinism in *Vitis* might be the result of two distinct sets of mutation in two linked gene regions, one including VSVV006 and VSVV007, and the other including VSVV009. As in *Fragaria virginiana* Mill. [58], the female and male sterile mutations could be not completely linked allowing the appearance of neuter and hermaphrodite individual. Some hermaphrodite grapevines have been already observed in natural conditions, but their wild status is still uncertain today as they may be escapees from cultivation [59]. Similarly, in the long-lived, late-flowering and disease-prone grapes, while non-flowering plants are observed both in the wild and in experimental breeding, it is very difficult to unequivocally establish whether these are neuter or just growing in flowering-limiting conditions.

The length of the small XY region in *Vitis vinifera* is less than 1% of the chromosome length, much shorter than the small sex region identified in papaya which covers 10% of the chromosome [60]. In this small sex region, the *flavin-containing monooxygenase* (FMO) genes and the *adenine phosphoribosyl transferase* (APT3) have been already suggested as good functional candidates for flower sex determination in grapevine [18]. Other candidate genes could be mentioned such as the *trehalose-6-phosphate phosphatase* (TPP) that controls inflorescence architecture in maize through sugar signal modification [61] and its direct product, the disaccharide trehalose, has a marked effect on flowering transition [62]. The *WRKY transcription factors* are one of the largest families of transcriptional regulators [63] and one of these factors has been shown to regulate endosperm growth and cellularization in *Arabidopsis* [64]. The VSVV008 amplicon was designed in a gene which the predicted protein reveals similarity with a *Ethylene Overproducer-like 1* (ETO1). The Arabidopsis ETO1 protein specifically inhibits the enzyme activity of the *1-aminocyclopropane-1-carboxylate synthase* (ACS) [65,66] known to be involved in sex determination in melons (*Cucumis melo*) [67]. However, for the *YABBY* protein, the polymorphisms did not correlate with phenotypic sex, suggesting that the association found by Battilana et al. [20] is the result of an

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extended intergenic linkage disequilibrium (LD), and not a direct indication of a causal mutation.

Origin of the H allele and traces of domestication

The last objective of this study was to elucidate the origin of hermaphroditism in domesticated grapevines. Both Fst and network analysis revealed that *H* haplotypes are more closely related to *M* than to *F* haplotypes. Thus, the *H* allele of the domesticated hermaphrodite grapevines may have derived from the *M* allele of wild male grapevines as suggested by previous authors [13,68]. Interestingly, in *Carica papaya,* hermaphrodites are also heterozygous for a Y chromosome variant (Y^h), more similar to the male-determining Y than to the X [60,69]. However, while all combinations of Y and/or Y^h are lethal in Papaya, in *Vitis HH* genotypes do strive and set seeds, as in the case of certain domesticated grapevines such as *Chardonnay*, *Muscat de Hambourg*, *Riesling* or *Cardinal* (Truel pers. comm., Vassal INRA), which produce 100% hermaphrodite progenies. Thus, the *H* allele may be an *M* allele having lost the dominant female sterility mutation, explaining the dominance of the *M* allele over the *H* allele. This hypothesis could also explain the increase in diversity observed in the *H* haplotypes as compared to the M haplotypes.

Studying phylogenetic patterns among the haplotypes, we could only found a weak tendency of the *H* macrohaplotypes of cultivars from Eastern regions cultivars, as compared to Western cvs, to be closer to the wild *M* macrohaplotypes. Former studies situated the major grapevine domestication region in the Eastern part of the Mediterranean area [9,53], which is thus consistent with our data.

More interestingly, the network analysis showed that both the *F* and *M/H* haplogroups are each divided in subgroups. In particular, wild female macrohaplotypes are subdivided in two main groups, one closely connected to the *V. balanseana F* haplotype, and the other farther away from *Vitis* sp. females; domesticated female haplotypes are divided in three groups, the first one close to the *V. balanseana* group, and the other two branching as independent lineages from the main *V. sylvestris* haplogroup. Similarly, in the *M/H* group, while the small differentiation within M haplotypes allows for less discrimination in the wild haplogroup, the cultivated hermaphrodites are again divided in groups, one including Eastern varieties and the other with a Western component. The general picture obtained with the network analysis points to a genetic structure of the wild *V. vinifera* haplotypes, in relation with other species, supporting the hypothesis presented in Peros et al. [25] that two chloroplast lineages from different Asian species (*V. piasezkii*, *V. amurensis* and *V. thunbergii*) contributed to the emergence of wild *V. vinifera* populations in Europe. On the other hand, the group differentiation in the domesticated compartment, both for the *F* and the *H* haplotypes, suggests multiple domestication events, as advanced by Arroyo et al. [11] based on chloroplast genetic diversity. More surprisingly, we found that the *H* haplotype from *cv. Assyl kara*, a Russian cultivar, derives directly, via a series of mutations, from *V. balansaena* (Figure 5). In the *F* group, the cultivar closest to *V. balanseana* is also a Russian cultivar, *cv. Ak ouzioum tapapskii*. Based on this evidence, we can advance the hypothesis that, in the sex region, in addition to the already known contribution from *V. vinifera* ssp. *sylvestris*, domesticated grapes enclose a genetic contribution from different Asian species. It is historically known that during the Soviet Union period, Russian agricultural researchers were active in importing genetic variability from diverse Asian regions as a source of cold or disease resistance alleles [70]. Indeed, Venuti et al. [71] recently showed that the Asian *Vitis amurensis* was used by breeders to introgress resistance genes into cultivated grapevines. However, since in our sample the *cv. Assyl kara* was recorded as one of the oldest traditional cultivar from North Caucasus [72], the introgression of a genetic contribution from Asian species into cultivated grapes may also significantly predate early 20th century breeding activities in Russia. It could well have occurred naturally through gene flow between different interfertile *Vitis* species followed by selection during domestication.

The very small differentiation found between the *H* and *M* haplotypes in the sex-linked amplicons and the small number of individuals studied here makes it difficult to clarify further the domestication pathway; this issue merits without doubt further exploration, reinforcing the argument of Venuti et al. [71] that new prospecting and collection of wild grapes and other *Vitis* species in the Eastern part of the domestication range are strongly needed presently.

The phylogeny position of *V. balansaeana*, *V. coignetiae* and *V. monticola* grapevines in our network, as well as segregation mapping in inter-specific crosses, both support a sex locus shared by all *Vitis* spp. [7,17], suggesting that the development of heteromorphic sex chromosomes is still in the very first stage of evolution in this taxon. In general, the age of a sex-determining region can be estimated from the age of the taxon in which it is found [23]. As in the subgenus *Vitis*, dioecy is the ancestral condition, its sex-determining region should be at least as old as the separation of the *Vitis* and *Muscadinia* subgenera, thought to have diverged approx. 18 My ago [57]. Other dioecious species with a sex region of approximately the same age, such as *Silene latifolia* [73], *Bryona dioica* [74] or *Rumex* spp. [75], have reached the final stages of sex chromosome evolution, with either full heteromorphic sex chromosomes or very large regions encompassing hundreds of genes. Future works to fully sequence the sex locus in a larger sample of genotypes in *Vitis* species

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could contribute to understand why some dioecious plants rapidly developed specific sex chromosomes, while others did not.

Conclusions

In *Vitis vinifera* subsp. *sylvestris*, we confirmed a sex locus of 151,8 kb located downstream to the marker VVIB23 and displaying haplotype diversity, linkage disequilibrium and differentiation that typically correspond to a small XY sex-determining region with XY males and XX females. This small sex-determining region, spanning less than 1% of chromosome 2 and also present in other *Vitis* species, suggests that grapevines could be organisms of choice to study the early stages of evolution of sex chromosomes in perennial species.

Hermaphrodite alleles appear to derive from male alleles of wild grapevines, with successive recombination events allowing import of diversity from the X into the Y chromosomal region and slowing the expansion of the region into a full heteromorphic chromosome. Macrohaplotypes network patterns are consistent with a major grapevine domestication region in the Eastern part of the Mediterranean area and secondary domestication events in geographically distinct areas. Finally, we hypothesise that in the sex region some domesticated grapes enclose a genetic contribution from different Asian species. Our findings should encourage new prospections and collection of wild grapes, including other *Vitis* species, in the Eastern part of the domestication range.

Availability of supporting data

The sequences data sets supporting the results of this article are available in the Genbank repository, [GenBank: KJ575622-KJ57662; http://www.ncbi.nlm.nih.gov/genbank/]".

Additional files

Additional file 1: Passport data of the genotypes studied in the different analyses. (*) short names used in haplotype trees and the network. Short name signification: c = cultivated; s = wild (sauvage), f = female, m = male, h = hermaprodite, hf or hh = hermaphrodites for which the genotype at the sex locus is predicted from to the study of the sex segregation in their progenies. (**): accessions codes from the INRA Vassal collection, France. (***) Geographic groups acronyms were defined as in Bacilieri et al. [35], namely: MAGH = Maghreb; IBER = Iberian Peninsula; WCEUR = Western & Central Europe; ITAP = Italian Peninsula; BALK = Balkans; RUUK = Russia & Ukraina; EMCA = Eastern Mediteranean and Caucasus; MFEAS = Middle and Far East; NEWO = New World; and ND = Non determined. (#) Geographic origin predicted from molecular evidences and a hierarchical clustering as in Bacilieri et al. [35].

Additional file 2: Primer sequences of the amplicons used in this study to cover the sex locus and its edges. (*) Gaze annotation. (**) Approximative values. (†) PN40024 reference sequence, 12×.0 version, amplicon position 16.072.323-16.073.307, Scaffold_233, chromosome UnRandom. (‡) PN40024 reference sequence, 8× version, amplicon position 5.192.572-5.193.382, scaffold 187, chromosome 2. (§) Primers developed in the gene predicted using the 8x Gaze annotation and confirmed by Fechter et al. [18] on the 12×.0 reference sequence version.

Additional file 3: Characteristics of the sequence polymorphisms found in the eleven amplicons covering the sex locus. For the Fisher tests, P-values and -log(p-value) in bold are the polymorphisms significantly deviating from the Hardy-Weinberg equilibrium. The underlined Fisher-test values correspond to significant polymorphisms following perfectly the XY sex-determination model : all male genotypes are heterozygous and all female are homozygous for the most frequent allele, i.e. for example males were A/T and females were A/A but never T/T. (*): the bold and italic position are approximate values; (**) F, M and Th = actual genotype count of females and males in the population, and theoretical proportions at Hardy-Weinberg equilibrium; he = heterozygote genotypes (for example AT). (***): ND = Not Defined.

Additional file 4: Maximum likelihood haplotypes trees of the four sex-linked gene fragments built to define the *M*, *F* and *H* haplogroups. Amplicons: a) VSVV006, b) VSVV007, c) VSVV009 and d) VSVV010. According to sex inheritance theory in *Vitis*, the *F* haplotypes regroup the *F* haplotypes of the *FF* females, *MF* males and *HF* hermaphrodites genotypes. Thus, the group containing haplotypes found in female, male and hermaphrodite genotypes was designated as the female *F* haplogoup. In green, haplotypes of domesticated hermaphrodite genotypes, in blue haplotypes of wild male genotypes and in pink haplotypes of wild female genotypes. The red dashed border box indicate the *F* haplogroup.

Additional file 5: Percentage of heterozygous genotypes in domesticated hermaphrodite grapevine. a) The gene amplicons' positions on the sex locus on the grapevine chromosome 2. b) percentage of heterozygous genotypes, at a given polymorphism, in hermaphrodite domesticated grapevines. The 14 genotypes considered here were expected to be heterozygous *HF* at the sex locus considering the sex segregation in their progenies. The red arrows indicate the polymorphisms perfectly linked to sex in male and female wild genotypes (see Figure 2 in the main document).

Additional file 6: Gene annotation of the sex region defined in the study (Gaze annotation 2012, 12x.0 version).

Additional file 7: Haplotypes of the four sex-linked amplicons. Haplotype representation for the four sex-linked amplicons ranked according to their inferred sex (from the haplotype trees) and the geographic origin of the genotypes. Haplotypic sex, *M* = male, *H* = hermaphrodite, *F wild* = female haplotype found in wild grapevine and *F dom* = female haplotype found in domesticated grapevine. Name: PHASE haplotype number. Individual: genotype name; short name signification: c = cultivated; s = wild (sauvage), f = female, m = male, h = hermaprodite, hf or hh = hermaphrodite for which the genotype is known (*HF* and *HH* genotype known through the study of the sex segregation in descents). Country: country of origin of the plant. Region: geographic groups acronyms were defined as in Bacilieri et al. [35], namely: MAGH = Maghreb; IBER = Iberian Peninsula; WCEUR = Western & Central Europe; ITAP = Italian Peninsula; BALK = Balkans; RUUK = Russia & Ukraine; EMCA = Eastern Mediterranean and Caucasus; MFEAS = Middle and Far East; NEWO = New World Vineyard; and ND = Not determined. Colors base representation: yellow = adenine, red = thymine, blue = cytosine and green = guanine.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

RB and JFT supervised the study and were PhD work coordinators for SP. SP, SI, DM, RB and JFT sampled plant material from natural populations. SP, SI and TL carried out plant phenotyping. TL provided plant materials coming from the Vassal collection. TL, RB and SP interpreted the statistical results with regard to the history of viticulture. SP, ML, MA and AW carried out DNA extractions and sequencing under the supervision of SS. SP and RB carried out the statistical calculations. SP and RB wrote the paper with the help and corrections of TL, PT, RAG, DM, PC and JFT. All authors read and approved the final manuscript.

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ORIGINAL ARTICLE

Allelic variation in the *VvMYBA1* and *VvMYBA2* domestication genes in natural grapevine populations (*Vitis vinifera* subsp. *sylvestris*)

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Abstract Grape skin color is among the most important qualitative traits on which selection is based in wine and table grape breeding programmes. Skin color is determined by the quantity and composition of anthocyanins. In prior work on cultivated forms, it was shown that polymorphisms in the grape transcription factor family *VvMYBA* are responsible for anthocyanin content variation in the berries of cultivated grapevine (*Vitis vinifera* subsp. *vinifera*). Wild grapevine (*V. vinifera* subsp. *sylvestris*) is the ancestor of the cultivated *V. vinifera* subsp. *sativa* and has black-colored berries. The purpose of this study was to determine how the *VvmybA1* and *VvmybA2* polymorphisms emerged and affected the genetic diversity of wild grapevines in the Mediterranean basin by examining samples from the Iberian Peninsula, Italian Peninsula and Caucasian region. Our observations provide evidence that variation in the two transcriptional regulators generated a novel allele series via length polymorphisms in *VvmybA1* and a point mutation in *VvmybA2*, which is lacking in cultivated grapevine. Further, correlation was detected between allele composition and anthocyanin contents. According to polymorphisms in both *VvMYBA* genes at the color locus, we were able to identify several haplotypes. The most ancestral haplotype (HapN) was found in wild grapevine in the western Mediterranean region and corresponded to wine grape cultivars, whereas recent haplotypes were detected in eastern regions. These eastern zones showed the most diverse haplotypes, which appeared in table cultivars where intense breeding practices may have replaced the original haplotype diversity. These findings provide information about the evolution of grapes since their domestication and have direct implications for wine quality.

Keywords MYB genes · Allelic variation · Cultivated grapevine · Wild grapevine · *Vitis vinifera* L.

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Introduction

Grapevine (*Vitis vinifera* L.) is the only species of the genus *Vitis* native to Eurasia. Today two forms of this species can still be identified: a wild form whose populations occur in river bank forests and have been assigned by taxonomists to *V. vinifera* subspecies *sylvestris*, and a cultivated form that groups cultivated varieties under the denomination *V. vinifera* subspecies *sativa* or *vinifera*. Most studies using nuclear genome molecular markers to

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compare *sylvestris* accessions with different sets of *vinifera* cultivars have shown that wild and cultivated genotypes often cluster in different genetic groups according to data from microsatellite markers (Grassi et al. 2003; Snoussi et al. 2004; Zinelabine et al. 2010; De Andres et al. 2012). Domestication leads to a genome-wide reduction in genetic diversity in crops relative to their wild progenitors due to genetic drift in the form of domestication bottlenecks (Meyer and Purugganan 2013). A recent large-scale survey of grapevine genetic diversity using microsatellites has estimated an average genetic diversity of 0.739 with an average of 9 alleles per locus for *V. vinifera* subsp. *vinifera* and 0.755 with an average of 9.5 alleles per locus for the subsp. *sylvestris* (De Andres et al. 2012). These genetic diversity values represent a high expected ratio of heterozygous loci in the genome, consistent with the limited domestication syndrome observed in grapevine (Myles et al. 2011).

During the process of domestication, numerous changes occur in the genetic and physiological make up of crop plants (Hancock 2004). However, in domesticated grapevine genotypes, certain morphological traits have practically become fixed, including hermaphrodite flowers, large clusters and large berries, along with higher sugar contents (Olmo 1995). Other grapevine domestication traits are not constitutive of the domestication syndrome because they are not fixed in cultivated genotypes. However, these have been selection targets for crop diversification by different researchers who have developed varieties with desirable visual or taste properties. Such is the case of berry color, which is fairly polymorphic among cultivated genotypes, contrary to the situation in wild populations (Fournier-Level et al. 2010).

Most variation in grape color can be explained by genetic variation in a single gene cluster of three MYB-type transcription factor genes designated *VvMYBA1*, *VvMYBA2*, and *VvMYBA3* (Fournier-Level et al. 2009). The white-skinned genotype arose as the result of disruption of the two functional MYB-related genes (*VvMYBA1* and *VvMYBA2*). In the case of *VvMYBA1*, gene silencing is produced by insertion of retrotransposon *Gret1*, whereas a single-nucleotide mutation in the *VvMYBA2* coding region leads to protein truncation rendering it non-functional (Walker et al. 2007). Several genetic studies have shown that white-skinned individuals are homozygous for the *VvmybA1a* allele, whereas colored-skinned individuals contain at least one copy of the functional *VvmybA1c* allele (Azuma et al. 2008; Kobayashi et al. 2004, This et al. 2007).

Several additional sequences of *VvMYBA1* have been reported. Walker et al. (2007) and Yakushiji et al. (2006) showed that skin color mutations in 'Pinot Noir' and 'Cabernet Sauvignon' from black-skinned to white-skinned, are caused by deletion of the functional *VvmybA1* allele. Yakushiji et al. (2006) named the null allele in 'Pinot Blanc' (white-skinned bud sport of 'Pinot Noir') *VvmybA1d*. Lijavetzky et al. (2006) observed *VvmybA1* expression in a white cultivar, 'Roditis', and found that the last 135 coding nucleotides of *VvmybA1* were deleted. These authors coined the name *VvmybA1ROD* for the allele that encodes the truncated protein. This et al. (2007) reported an additional 44-bp insertion within the promoter region of *VvmybA1* in several cultivars, and mentioned that the insertion was frequently related to red- and pink-skinned cultivars called *VvmybA1b*. Further, a double insertion of 111- and 44-bp was observed within the promoter region of *VvmybA1,* called $VvmybA1^{SUB}$ in other cultivars (Lijavetzky et al. 2006; This et al. 2007). Azuma et al. (2008) detected an unknown fragment other than *VvmybA1a*, *VvmybA1b*, or *VvmybA1c* in colored-skinned cultivars of *V. labruscana* such as 'Concord' using allele-specific PCR primers for *VvmybA1*, but were unable to detect the fragment in any *V. vinifera* cultivars or in white-skinned cultivars of *V. labruscana* (Kobayashi et al. 2004). However, it is not clear whether the putative new allele represented by this fragment regulates anthocyanin biosynthesis, as do *VvmybA1b* and *VvmybA1c*.

For the two polymorphisms shown to be functionally responsible for berry color variation (Kobayashi et al. 2005; Walker et al. 2007), namely *Gret1* in *VvMYBA1* and K980 in *VvMYBA2*, four 'haplogroups' have been defined according to the presence or absence of the *Gret1* retroelement in the *VvMYBA1* promoter, and the presence of a functional G allele or a mutated T allele at the SNP K980 in the *VvMYBA2* coding sequence (Fournier-Level et al. 2010). Because these genes occurring within each copy of the color locus are inherited together, it is helpful to consider them as part of a single haplotype. In effect, Fournier-Level et al. (2010) reported that haplotype (Hap) C can be divided into two subgroups (haplogroups) called HapC-N, containing the functional *VvmybA1c* and *VvmybA2r* alleles, and HapC-Rs containing the functional *VvmybA1c* allele and the non-functional *VvmybA2w* allele along with haplotype B containing the non-functional alleles of *VvMYBA1* and *VvMYBA2*.

The manner in which these mutations emerged and affected genetic diversity in cultivated grapevine has been examined by Fournier-Level et al. (2010). However, analysis of the ancestor of the domesticated grapevine should improve our understanding of the evolution of domesticated traits in grapevine. The present work is the first survey designed to characterize allelic variation in *VvMYBA1* and *VvMYBA2* genes in 318 wild grapevine accessions from the Mediterranean basin. To characterize ancestral haplotypes in the color locus of wild grapevine accessions, herein we examine genetic variation in *VvMYBA1* and *VvMYAB2* loci. Our results reveal new

alleles produced by length polymorphisms in *VvMBYA1* and a point mutation in *VvMYBA2*, which has not been found in cultivated grapevine. Finally, we discuss nucleotide polymorphisms of these genes in cultivars and wild accessions and examine haplotype diversity in the Mediterranean basin.

Materials and methods

Plant material

The plant material examined comprised 318 wild accessions (*V. vinifera* subsp. *sylvestris*) from Spain (Iberian Peninsula IP, 192 genotypes), Italy (Italian Peninsula ITP, 82 genotypes), and Georgia (South Caucasian region CAU, 44 genotypes) (Online Resource 1, 2). When collecting the *sylvestris* samples, we sampled current relict populations with few individuals per population and, in most cases, obtained all present individuals. The current locations of these populations are not close to actual vineyards. To identify wild grapevine forms and avoid sampling possible naturalized cultivars or rootstocks, we followed strict genetic and morphological criteria, as reported in De Andres et al. (2012) for IP wild populations, Biagini et al. (2012) for ITP wild populations and Imazio et al. (2013) for CAU *sylvestris* samples.

PCR analysis of *VvMYBA1* gene structure

Genomic DNA was isolated from young leaves using a DNeasy Plant Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. The *VvmybA1* alleles were first scored using a PCR assay originally based on amplification of the 5′ flanking region and coding sequences of the insertion *Gret1* of *VvMYBA1* and its functional reversion allele, denoted *VvmybA1a* and *VvmybA1c*, respectively (Kobayashi et al. 2004). The primers used for functional allele amplification were F2 described by Azuma et al. (2008) and R1 described by Lijavetzky et al. (2006). PCR reactions were performed as suggested by Azuma et al. (2008) and PCR fragments separated by electrophoresis in 1.5 % agarose gel in TBE buffer and photographed under UV light.

Cloning of *VvmybA1* alleles

PCR amplification to isolate the *VvmybA1* upstream region was performed using the F2 (Azuma et al. 2008) and R1 (Lijavetzky et al. 2006) primers as described above. PCR fragments were separated by electrophoresis in 0.8 % agarose gel in TBE buffer and purified using the Geneclean Turbo Kit (MP Biomedicals, Santa Ana, California). The purified fragments were ligated in the pGEM®-T Vector System I (Promega, Madison, Wisconsin) and used to make an *E. coli* plasmid library according to the manufacturer's instructions. The selected plasmid sequences were purified by liquid culture using the Plasmid Mini Kit I (Omega Bio-Tek, Norcross, Georgia) and loaded onto an ABI PRISM® 310 Genetic Analyzer sequencer at the Genomics Unit of the Parque Científico de Madrid (Madrid, Spain). Sequence analysis and alignments were performed using the programme BIOEDIT v7.0.5.3 (Hall 1999). Sequences were compared with 10 cultivated genotypes described previously (Online Resource 1).

SNaPshot assay and sequencing of *VvMYBA2*

To investigate sequence polymorphisms in *VvmybA2* alleles, 10 IP wild grapevine accessions characterized only by their low anthocyanin contents were sequenced at the *VvmybA2* locus (Online Resource 3). The *VvMYBA2* gene sequence was amplified using the primers and conditions reported in Walker et al. (2007). PCR fragments were separated by electrophoresis in 0.8 % agarose gel in TBE buffer, purified using the Geneclean Turbo Kit and cloned (see 'Cloning of *VvmybA1* alleles' section for more details).

The *VvmybA2* sequences obtained served to identify a new point mutation designated *VvmybA2C22*. This polymorphism was assessed in the 318 wild specimens using the SNaPshot method, along with the single-nucleotide polymorphism (SNP) *VvmyA2R44* (*VvMYBA2w* allele or K980; Walker et al. 2007). The primers to detect SNPs were designed up stream, contiguous to the polymorphic base. The sequence of the SNP was AGGATGTT CTCCTGAGGAAA and the native *VvmybA2w* sequence was GCAGGGTTGAATAGATGCC; both primers were HPLC purified.

The *VvMYBA2* gene sequence was amplified using the primers and conditions reported in Walker et al. (2007). PCR fragments were separated by electrophoresis in 0.8 % agarose gel in TBE buffer and purified using the Geneclean Turbo Kit according to the manufacturer's instructions. SNP genotyping was detected by the SNaPshot assay according to the protocol provided in the ABI PRISM SNaPshot Multiplex kit (Life Technologies Corporation, Carlsbad, California). The SNaPshot PCR products were enzymatically treated with 1 U each of alkaline phosphatase and calf intestinal alkaline phosphatase (CIP; New England Biolabs, Ipswich, Massachusetts) to degrade excess PCR primers and dNTPs. The reaction solution was mixed thoroughly and incubated at 37 °C for 1 h, followed by 15 min at 75 °C to inactivate the enzymes. The purified SNaPshot PCR products were detected on a capillary

electrophoresis instrument (ABI PRISM® 310 Genetic Analyzer) and data analysis was performed using GeneMapper 4.0 software.

Molecular data analysis

The VvmybA1 and VvmybA2 loci of 20 of the IP and CAU accessions were sequenced (Online Resource 4). DNA sequences were analyzed using the Staden Package (Bonfield et al. 1995). Heterozygous SNPs were identified as double pics on the chromatograms and coded according to international codes (Cornish-Bowden 1985). Haplotype reconstructions were conducted using Phase software (Stephens and Scheet 2005) and the SNiPlay web-based tool for SNP and polymorphism analysis (Dereeper et al. 2011). Haplotype diversity (Hd), nucleotidic diversity (Pi), and their error estimates were calculated for each gene pool using DnaSP4.0 software (Rozas et al. 2003). To test for departures from the standard neutral model of evolution, we used Tajima's D test (Tajima 1989). Statistics were computed to obtain insight into the hypothesis of selective neutrality. A non-significant value indicates no evidence of evolutionary selection. The above analyses were performed using DnaSP4.0 software (Rozas et al. 2003).

Relationship between haplotype composition and grape skin color

Total anthocyanin contents of the berry skin of 117 female wild grapevine accessions from the Iberian Peninsula were determined according to the method of Revilla et al. (2010) (Online Resource 5). Grape skins were ground in a Kinematica PCU-2 blender for 1 min, and subjected to sequential extraction with different solvents (methanol for 16 h at −25 °C, 80 % methanol for 4 h at room temperature, 50 % methanol for 4 h at room temperature, deionized water for 16 h at −25 °C, and 75 % acetone for 1 h at room temperature) using 25 mL of solvent for each extraction step, as described elsewhere (Revilla et al. 1998, based on Bourzeix et al. 1986). For each sample, all the liquid extracts were combined and then stored at 4 °C prior to their analysis. Total anthocyanin concentrations in grape extracts were determined by visible spectrophotometry (Niketic-Aleksic and Hrazdina 1972) using an ND-1000 Thermo Fisher Scientific spectrophotometer (Waltham, MA, USA). These contents were expressed as mg of cyanidin-3-glucoside (Extrasynthèse, Genay, France) equivalent per kilogram of fresh berry skin weight. The relationship between haplotypes at the berry color locus and total anthocyanin contents was assessed using Fisher's protected LSD test.

Results

MYB-related gene structure at the color locus

In this survey, we examined allelic diversity at the color locus (genes *VvMYBA1* and *VvMYBA2*) in 318 wild grapevine accessions from the Mediterranean basin (Online Resource 1). Six different functional and non-functional alleles of the *VvMYBA1* gene were identified (Fig. 1). Our results indicate that most of the wild grapevine accessions examined carried the wild-type allele *VvmybA1c* (Fig. 1). Moreover, in the cultivated grapevine group, we detected the functional $VvmybA1^{SUB}$ allele (Shimazaki et al. 2011), which is 188 bp longer than the wild-type allele due to three insertions (44, 111, and 33 bp), the allele with a single 44-bp insertion in the promoter region of the *MYBA1* gene *VvmybA1b* (This et al. 2007; Azuma et al. 2008; Shimazaki et al. 2011) and two new alleles, not detected previously. We have designated one of the new alleles *VvMybA1e* (Genbank accession KJ739718); this allele features a single 111 bp insertion in the promoter region and the 33 bp insertion in the second intron of the *MYBA1* gene (Fig. 1). The other allele has a small 55-bp deletion in the promotor region and we have called it *VvMybA1f* (Genbank accession KJ56341) (Fig. 1). These alleles were only present in wild-type samples of the Iberian Peninsula and Caucasian populations, respectively. The non-functional allele, *VvmybA1a*, was also detected. By examining this large pool of *V. vinifera* subsp. *sylvestris* genotypes ($n = 318$), we were able to observe the presence in heterozygosity of *Gret1* in wild accessions from the Mediterranean basin. The percentage of this non-functional allele *VvmybA1a* was significantly higher in the Italian (81 %) and Georgian populations (71 %) than the Spanish population (12 %) (Table 1).

When we assessed *VvMYBA2* gene polymorphisms, two SNPs were examined: the non-functional allele *VvmybA2w*

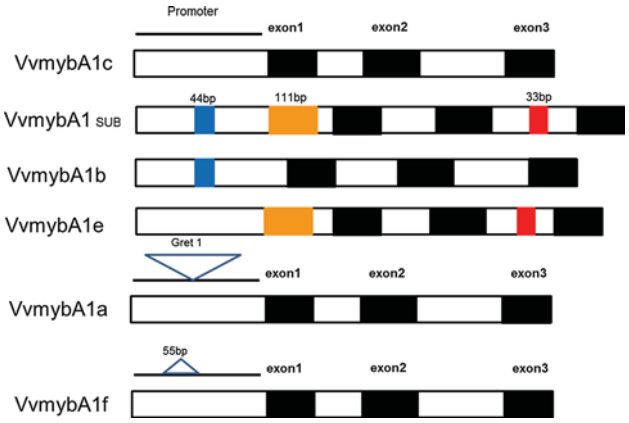

Fig. 1 *VvMYBA1* gene structure in wild grapevines

Table 1 Allele frequencies for both *VvMYBA* genes in the wild grapevine accessions ($N = 318$) from the Mediterranean basin examined in this study

Region (no. samples)	*VvMYBA1*						*VvMYBA2*			
Samples	VvMYBA1c	VvMYBA1SUB	VvMYBA1b	VvMYA1e	VvMYA1f	VvMYBA1a	K44 wild	K44 mutated	C22 wild	C22 mutated
IP (192)	0.895	0.05	0.005	0.05		0.12	0.82	0.18	0.95	0.05
ITP (82)	0.05	0.95				0.81	0.9	0.1	0.93	0.07
CAU (44)	0.56	0.35		0.07	0.02	0.71	0.75	0.25	0.89	0.11

called *VvmybA2R44* or K980 discovered by Walker et al. (2007) and a new SNP detected here by sequencing 10 wild grapevine accessions with low anthocyanin contents (Online Resource 3) called *VvmybA2C22*. By sequencing these accessions, we were able to identify the point mutation (K980) described by Walker et al. (2007) and the new SNP. The new polymorphism consists of a different residue sequence in the α-helix protein-coding region and differences at the protein level indicated a predicted non-conservative amino acid change from cysteine at position 22 to the non-synonymous glycine. Recently, Feller et al. (2011) reported conservation of the cysteine residue in the alignment of MYB regulators of anthocyanin biosynthesis in all seven dicot species of nine different plant species. This conservation suggests that the cysteine residue is functionally important for the binding of DNA or other proteins.

Allelic diversity of the *VvMYBA1* and *VvMYBA2* genes in natural populations

The frequencies of the functional and non-functional alleles of the *VvMYBA*1 and *VvMYBA2* genes detected in the 318 accessions from the Mediterranean basin are provided in Table 1. The most frequently occurring functional alleles were the wild-type *VvmybA1c* and $VvmybA1^{SUB}$ alleles. The frequency shown by the *Vvmyb1c* allele (89 %) in the wild accessions from IP was higher than in those from CAU (56 %) (Table 1). The allele $VvmybA1^{SUB}$ (95 %) emerged as the most frequent allele in the wild accessions from ITP followed by the CAU samples (35 %). The alleles *VvmybA1b*, *VvmybA1e*, and *VvmybA1f* are minor alleles: *VvmybA1b and VvmybA1f* alleles are present only in the IP and CAU, respectively, and the *VvmybA1e* allele is present in CAU and IP (Table 1). To examine allelic diversity in the *VvMYBA2* gene, we determined the presence of two mutations in the wild grapevine accessions. Using the SNaPshot system (Applied Byosystem, CA), we were able to detect the new nucleotide polymorphism *VvmybA2C22* and the SNP *VvmybA2R44* (Walker et al. 2007). The allelic frequencies of both SNPs are provided in Table 1. The most frequent allele was the wild-type allele of *VvMYBA2* followed by the non-functional allele *VvmybA2R44* (Walker et al. 2007). The new mutated allele *VvmybA2C22* showed low frequency in all the samples analyzed (Table 1).

Sequence diversity in the *VvMYBA1* and *VvMYBA2* genes in wild *V. vinifera* accessions from Spain and Georgia

The *VvMYBA1* and *VvMYBA2* genes were directly sequenced on a core collection of 20 wild accessions from both extremes of the Mediterranean basin (10 samples from IP and 10 from CAU), which represented the maximum allelic diversity of this gene pool (Online Resource 4). The DNA fragments of the two genes correspond to the isogenes GSVIVT000226590001 and GSTVIVT00022658001, respectively. For *VvMYBA1*, we obtained an amplicon of 1,035 bp, while for *VvMYBA2* an amplicon of 1,240 bp was recovered. Sequence diversities for each gene region were compared with those of 10 published cultivated genotypes (Fournier-Level et al. 2010). Direct DNA sequencing of *VvmybA1a* from the wild genotypes revealed insertion of *Gret1* at the same position within the promoter *VvMybA1* (data not shown).

Through sequence analyses of the functional genes *VvMYBA1* and *VvMYBA2* in the wild genotypes, polymorphic sites were identified within the fragment comprising the promoter region, as well as the exons and the introns of these genes. Sequencing of 1,035 bp of the *VvMYBA1* gene for a sample consisting of 20 wild and 10 cultivated grapevine accessions led to the detection of 20 bi-allelic SNPs (Table 2). Among the 20 SNPs, 5 SNPs were located in the coding region; of these 3 were synonymous and 2 non-synonymous giving rise to a change in the encoded amino acids. Further, among the detected SNP set, 12 SNPs were localized in the promotor region. For the gene *VvMYBA2*, sequencing of 1,240 bp in 20 wild and 10 cultivated grapevine accessions revealed 27 SNPs (Table 3). Of these 27 SNPs, 3 were located in the coding region: one synonymous and two non-synonymous,

Table 2 Features of SNPs detected in the gene *VvMYBA1*

SNP	Relative ATG position (PN sequence)	PN allele	Wild-type allele	Feature	Amino acid change
M51	−255	A	C	Promotor	a
S59	−248	G	C	Promotor	a
K77	−230	T	C	Promotor	a
Y188	−163	T	C	Promotor	a
W191	−160	A	T	Promotor	a
Y204	−147	T	C	Promotor	a
Insertion213	−138	–	G	Promotor	a
K234	−118	T	G	Promotor	a
M242	−110	A	C	Promotor	a
R433	−30	G	A	Promotor	a
Y438	−25	C	T	Promotor	a
K462	−1	G	T	Promotor	a
Y497	+35	T	C	Exon	I11 → T
R529	+67	A	G	Exon	I23 → V
W617	+145	T	A	Intron	a
W619	+157	T	A	Intron	a
R647	+185	A	G	Intron	a
R793	+231	G	A	Exon	R48
R714	+252	G	A	Exon	L55
Y764	+297	T	C	Exon	D70

The VvmybA1c allele appearing in the wild grapevine accessions relative to the same allele in Pinot Noir

[a] No change

Table 3 Features of SNPs detected in the gene VvMYBA2

SNP	Relative ATG position (PN sequence)	PN allele	Wild-type allele	Feature	Amino acid change
R31	−711	G	A	Promotor	a
R59	−683	A	G	Promotor	a
S69	−673	C	G	Promotor	a
Y140	−602	C	T	Promotor	a
K153	−589	T	G	Promotor	a
K181	−561	T	G	Promotor	a
R182	−560	G	A	Promotor	a
Y194	−548	T	C	Promotor	a
Y212	−530	C	T	Promotor	a
R337	−405	A	G	Promotor	a
M403	−339	C	A	Promotor	a
Y420	−322	T	C	Promotor	a
K438	−304	T	G	Promotor	a
K449	−293	T	G	Promotor	a
R485	−257	A	G	Promotor	a
508	−234	T	–	Promotor	a
509	−233	G	–	Promotor	a
S582	−160	C	G	Promotor	a
K587	−155	G	T	Promotor	a
Y738	−4	C	T	Promotor	a
K805	63	T	G	Exon	C22 → G
R859	118	A	G	Intron	a
R877	136	A	G	Intron	a
R887	146	G	A	Intron	a
900	160	–	T	Intron	a
R960	219	G	T	Exon	R44 → L
R972	231	G	A	Exon	R48

The VvmybA1c allele appearing in the wild grapevine accessions relative to the same allele in Pinot Noir

[a] No change

causing a change in the encoded amino acids. One of these non-synonymous SNPs was described by Walker et al. (2007) and the other one was a new point mutation K805 we refer to as *VvmybA2C22*. In addition, among the detected SNP set, 21 SNPs were localized in the promotor region.

All indicators of genetic diversity (number of polymorphic sites, number of haplotypes, haplotype diversity, and nucleotide diversity) were used to characterize and compare diversity levels in the analyzed gene pools (Table 4). The CAU wild accessions revealed 23 SNPs in gene *VvMYBA1* rendering 5 haplotypes, whereas 18 SNPs giving rise to 6 haplotypes were observed for gene *VvMYBA2*. Haplotype diversity (Hd) varied from 0.9333 ± 0.01488 (*VvMYBA1*) to 0.972 ± 0.00041 (*VvMYBA2*) and nucleotide diversity (Pi) from 0.0137 ± 0.0026 (*VvMYBA1*) to 0.00749 ± 0.0014 (*VvMYBA2*). Similar results were obtained for the IP wild accessions; 19 SNPs were detected for this population in the *VvMYBA1* gene, giving rise to 9 haplotypes, and 17 SNPs were detected in *VvMYBA2*, generating 8 haplotypes. Haplotype diversity ranged from 0.964 ± 0.0026 (*VvMYBA1*) to 0.972 ± 0.0004 (*VvMYBA2*) and nucleotide diversity (Pi) from 0.009 ± 0.002 (*VvMYBA1*) to 0.006 ± 0.0011 (*VvMYBA2*). These findings indicate similar haplotype diversity in both genes in these wild genotypes yet higher nucleotide diversity (Pi) in *VvMYBA1* than *VvMYBA2*. Comparable results have been obtained for Tunisian wild populations (Riahi et al. 2013).

Our data for 10 European grapevine cultivars revealed significant lower haplotype diversity values for *VvMYBA1* (0.867 ± 0.007) compared to the values obtained for the wild IP (0.964 ± 0.002) and CAU samples (0.933 ± 0.014) (Table 4). When we examined haplotype diversity in the *VvMYBA2* gene, the European cultivars showed similar values to those recorded for the wild CAU and IP populations. In summary, nucleotide diversity in the *VvMYBA* genes was similar in all the samples analyzed,

Table 4 Pattern of nucleotide diversity observed for the two *VvMYBA* genes in wild grapevines from both extremes of the Mediterranean basin compared with the European grapevine cultivars

Samples	Region (bp)	NPS	NH	Hd	VarHd	Pi	VarPi	Tajima's D value
A								
VvMYBA1								
W European								−1.586
Cultivars ($N = 10$)	1–1,035	17	6	0.867	0.00723	0.01041	0.00149	
Wild								0.359
IP grapevines ($N = 10$)	1–1,035	19	9	0.964	0.0026	0.00928	0.00182	
Wild								0.256
CAU grapevines ($N = 10$)	1–1,035	23	5	0.933	0.01481	0.01371	0.0026	
B								
VvMYBA2								
W European	1–1,240	22	19					−3.524*
Cultivars ($N = 10$)				0.952	0.00022	0.00637	0.00049	
Wild IP	1–1,240	17	8					0.635
Grapevines ($N = 10$)				0.972	0.000409	0.00626	0.00119	
Wild CAU	1–1,240	18	6					0.589
Grapevines ($N = 10$)				0.972	0.00409	0.00749	0.0014	

Nucleotide diversity for *VvMYBA1*. The neutrality test of Tajima for *VvmyA1* was not significant $p > 0.01$

NPS Number of polymorphic sites, *NH* number of haplotypes, *Pi* nucleotide diversity, *Hd* haplotype diversity, *IP* Iberian Peninsula, *CAU* caucasus

* Nucleotide diversity for *VvMYBA2*. The neutrality test of Tajima was significant $p < 0.01$

though generally slightly higher values were observed for the wild-type samples.

One of the main applications of knowledge of SNP diversity is to assess the evolutionary factors that may have affected specific loci. To detect possible signatures of selection in the loci examined, we used the Tajima's D neutrality test. Results indicated non-significant differences among the wild-type grapevine accessions. However, significant negative values were recorded in the cultivated grapevine samples for the gene *VvMYBA2* (Table 4). Similar results have been described by Riahi et al. (2013).

Haplotype composition and diversity in natural populations from the Mediterranean basin

The *VvMYBA1* and *VvMYBA2* genes are located within each copy of the color locus and are inherited together such that they are often considered as part of a single large locus (haplotype). Recently, Fournier-Level et al. (2010) reported HapN as the major haplogroup in cultivated forms containing the functional alleles for *VvMYBA1* and *VvMYBA2*. In contrast, HapRs contains the functional allele of *VvMYBA1* and non-functional allele at position R44 of *VvMYBA*2 and HapB contains the homozygous non-functional alleles in both genes. Finally, the HapF described by Shimazaki et al. (2011) shows the double insertion (44 and 111 bp) in the promoter region of *VvMYBA1* and the 33-bp insertion in the second intron region (Table 5). We investigated the haplotype composition of the wild gene pool and observed the same haplotypes as in the cultivated sample group but detected additional haplotypes. The new haplotypes detected in the wild accessions were HapC, D, E, G, and H (Table 5): HapC carried the double insertion of the promoter region of *VvMYBA1* (44- and 111-bp insertions) and the new mutation C22 in the *VvMYBA2* gene; HapD only showed the new point mutation allele at C22 in the *VvMYBA2* gene; HapE carried the 55-bp deletion in the promotor region of the *VvMYBA1* gene; HapG showed a triple band corresponding to a double 111-bp/44-bp insertion plus a single 111-bp insertion in the promoter region of *VvMYBA1*; and, finally, HapH only carried the single 44-bp insertion in the promoter region (Table 5). The homozygous non-functional haplotype B plants ware not detected in our samples.

Our study compares the haplotype compositions of grapevine populations across the Mediterranean basin. HapN was identified as the most frequent haplotype in the IP wild accessions and Spanish autochthonous cultivars. In the ITP samples, the most common haplotype was HapF. In contrast, the CAU accessions showed more diverse haplotypes including the high frequency of HapN and F (Table 6).

Haplotype composition at the color locus was then correlated with anthocyanin content in the Spanish wild grapevine populations. All 117 Spanish female plants analyzed showed colored grape skin. The plants were divided into those homozygous for the functional haplotype N ($n = 75$) and those heterozygous for haplotype N and the non-functional haplotype B ($n = 41$), and mean anthocyanin contents determined for each group (Fig. 2). Mean anthocyanin contents were significantly higher for the homozygous than the heterozygous functional genotypes (Fig. 2). These results suggest that the number of functional alleles affects the plant's capacity to accumulate

Table 5 Haplotype composition of the *VvMYBA* genes in the wild grapevine accessions ($N = 318$) examined in this study

Haplotype composition	*VvMYBA1*						VvMYBA2		
	VvmybA1SUB	*VvmybA1c*	*VvmybA1b*	*VvmybA1e*	VvmybA1f	R44wild	R44 mutated	C22wild	C22 mutated
Hap N (Fournier-Level et al. 2010)	−	+	−	−	−	+	−	+	−
Hap Rs (Fournier-Level et al. 2010)	−	+	−	−	−	−	+	+	−
Hap F (Shimazaki et al. 2011)	+	−	−	−	−	+	−	+	−
Hap C	+	−	−	−	−	+	−	+	
Hap D	−	+	−	−	−	+	−	−	+
Hap G	+	+	−	+	−	+	−	+	−
Hap H	−	−	+	−	−	+	−	+	−
Hap E	−	−			+	+	−	+	−

+ indicated present allele and – absent allele

Table 6 Haplotype frequencies detected in the *VvMYBA* genes in wild grapevine accessions from the Mediterranean basin ($N = 318$)

No. of individuals Haplotype composition	44 CAU	82 ITP	192 IB
Hap N (Fournier-Level et al. 2010)	0.4		0.89
Hap Rs (Fournier-Level et al. 2010)	0.07	0.05	0.02
Hap F (Shimazaki et al. 2011)	0.36	0.9	0.005
Hap C	0.02	0.04	
Hap D		0.01	0.04
Hap G	0.13		0.04
Hap H			0.005
Hap E	0.02		

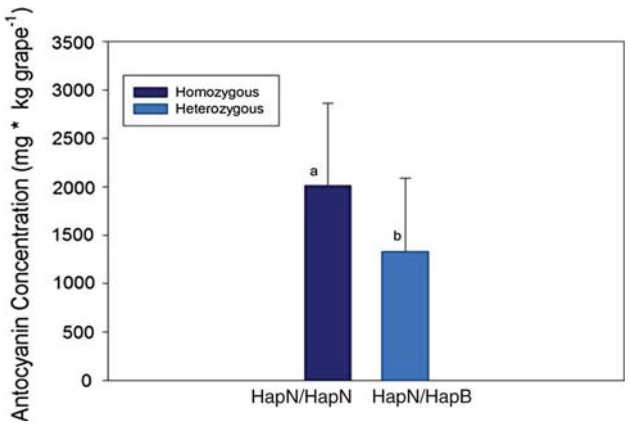

Fig. 2 Relationship between haplotype composition at the color locus and total anthocyanin contents in berry skins from wild grapevine accessions from the Iberian Peninsula

anthocyanin in grape berry skin. Similar observations have been reported by others (Fournier-Level et al. 2010; Azuma et al. 2011).

Discussion

Recent interest in crop evolution studies has focused on identifying genes that control phenotypes of biological and agronomic significance. On the basis of an increase in the nucleotide substitution rate, it has been proposed that morphological evolution proceeds via diversification in regulatory loci (Meyer and Purugganan 2013). Polymorphisms in the grape transcription factor *VvMYBA* are known to be responsible for variation in the anthocyanin contents of the berries of cultivated grapevine (Fournier-Level et al. 2010). This study was designed to gain insight into how these mutations emerged and their effects on wild grape and cultivated grapevine populations.

Genome analysis of wild grapevine accessions revealed sequence polymorphisms of the *VvMYBA1* gene. These polymorphisms included 44-bp and 111-bp insertions in the promotor region, also reported by other authors (Lijavetzky et al. 2006; Azuma et al. 2008; Shimazaki et al. 2011). These insertions in the *MYBA1* promoter seem to be not functionally linked to grape skin color. However, a single 44-bp insertion has been associated with red or pink grape berries (This et al. 2007) and other *Vitis* species with the 44-bp insertion such as *V vinifera* × *V. labruscana* have black berries (Azuma et al. 2008). Recently, Shimazaki et al. (2011) observed that the double 44-bp/111-bp insertion was able to spread to black berry *Vitis* species, namely, *V. labrusca*, *V. rupestris*, *V. riparia*, *V. coignetiae*, and *V. amurensis*. These insertions were present in the wild accessions from the Mediterranean basin examined here although the alleles were found to occur at a higher frequency in the natural Italian and the Caucasian populations. In addition, we detected a single 111-bp insertion in the promotor region of *VvMYBA1* in the IP and CAU wild grapevine populations. This allele has not been previously

described and it seems that the insertion is not associated with grape color variation, because the berries are black. Thus, both the 44- and 111-bp insertions do not behave as determinants of berry color. The functional significance of these insertions in the promotor region of *MYBA1* remains unclear. The 33-bp insertion in the second intron region of *MYBA1* in the *VvmyA1*^{SUB} and *VvmybA1e* alleles has also been described in cultivated grapevine populations by three other research groups (Lijavetzky et al. 2006; Azuma et al. 2008; Shimazaki et al. 2011). Shimazaki et al. (2011) found that pink colored oriental *V. vinifera* cultivars have the 33-bp insertion in the second *VvMYBA1* intron without exception. Also, it has been shown that the 33-bp insertion affects the splicing and maturation of the MYBA1 transcript resulting in the low expression of MYBA1 in grape berry skin (Shimazaki et al. 2011). Consequently, unspliced MYBA1 may be degraded by an unknown mechanism leading to suppressed anthocyanin biosynthesis due to reduced MYBA1 protein in the skin. We here detected the 33-bp insertion in the wild gene pool at a significantly higher frequency in the natural Italian and the Caucasian populations, although it was not related to color variation. In addition, a unique allele showing a 55-bp deletion in the promoter region was detected in the wild CAU samples. The function of this new allele is unknown because it always appeared in a heterozygous state. Considering that western *V. vinifera* cultivars, such as Cabernet Sauvignon, Pinot noir, and Merlot, never have the short 33-bp insertion in their second *MYBA1* intron as well as the 44- and 111-bp insertions in their *MYBA1* promoter, these insertions provide information about the evolution and domestication of grapes. Our results suggest that the alleles at the color locus described for the western *vinifera* cultivars occur in most western wild accessions. In addition, the *orientalis* cultivars featured the same alleles as the central and eastern wild accessions; although in both groups, we detected some ancestral wild-type alleles not present in the cultivars. These observations are consistent with the phenotypic and genetic differences described for cultivars from both extremes of the Mediterranean basin. According to Negrul (1938), morphotype classification of cultivated grapes serves to distinguish an *occidentalis* group, characterized by the small berry grapes of Western Europe, an *orientalis* group comprised the large berry cultivars of central Asia, and a *pontica* group including the intermediate types from the Black Sea basin and Eastern Europe. Using SSR markers V. *vinifera* cultivars from *orientalis* and *occidentalis* proles have been also differentiated (Aradhya et al. 2003; Arroyo-Garcia et al. 2006). Although we cannot discard that the presence of the same alleles in the color locus of wild and domesticated compartments, it is due to gene flow between both compartments or ancestral polymorphisms persisting in heterozygous condition in wild grapevine.

In our study, the white allele, which included the retrotransposon insertion in the promoter region, appeared in both cultivated and wild grapevine genotypes. *VvmybA1a* (which contains the *Gret1* insertion) has been detected in many accessions of *V. vinifera* and *V.* x *labruscana*, but was not observed in any of the North American or East Asian *Vitis* species tested (Mitani et al. 2009). Our results suggest that white alleles originated in *V. vinifera* subsp. *sylvestris,* the ancestor of the cultivated grapevine although we cannot exclude the possibility that the presence of this allele in the wild-type group is due to hybridization with the cultivated group. At the genotype level, crop evolution has favored an excess of heterozygous genotypes, with the emergence of functional and non-functional alleles that spread extremely rapidly. Although the *Gret1* insertion has been considered the main factor determining grape color (Kobayashi et al. 2005; This et al. 2007), the K980 mutation of *VvMYBA2* appears to have played an essential role in the diversification of *VvMYBA*. This mutation was here observed in the ancestral group at moderate frequency and we were able to identify a new *VvMYBA2* mutation in the wild-type gene pool that was probably not selected for during the domestication process. The findings of the present study thus indicate that the allele structure of the color locus in *V. vinifera* subsp. *sylvestris* differs from that in *V. vinifera* subsp. *vinifera*.

Nucleotide diversity was higher for the *VvMYBA1* than *VvMYBA2* gene. In total, 20 SNPs were detected in the sequenced *VvMYBA1* fragments giving an average of 1 SNP every 51 bp. This level of polymorphism is in general higher than previously reported for grapevine cultivars in other studies, including averages of 1 SNP every 64 bp (Lijavetzky et al. 2007), or even one every 100 bp (Velasco et al. 2007). However, in other nucleotide diversity studies examining wild grapevine, mean SNP frequencies of one SNP every 33 bp (Riahi et al. 2013) or one every 23 bp (Dong et al. 2010) have been detected. This confirms a high rate of polymorphism in grapevine compared with other plant species. The high diversity of grapes is hardly coherent with the reduced number of generations since domestication, which is thought to be under 100 (Fournier-Level et al. 2010). Another source of such high DNA polymorphism could be hybridization with cultivated grapevine (Di Vecchi-Staraz et al. 2009; De Andres et al. 2012). Since domestication, cultivated grape has evolved in sympatry with its wild relative, *V. vinifera* L. subsp. *sylvestris*, and hybridization is likely to have occurred. In a genetic diversity survey of wild grapevines, gene flow estimates between wild and cultivated grapes indicated the existence of hybrids but at a low frequency (Di Vecchi-Staraz et al. 2009; De Andres et al. 2012; Arroyo-García and Revilla 2013). The phylloxera crisis at the end of the nineteenth century led to a drastic reduction in population

size (Levadoux 1956) and today's recovered genetic diversity may not reflect the diversity present in the ancestral gene pool.

Among the SNPs detected here, most occurred in the non-coding region as reported in most studies. Riahi et al. (2013) described that in 67 % of cases, mutations in the coding regions of *VvMYBA1* and *VvMYBA2* are non-synonymous causing variation in the encoded amino acids. This suggests the contribution of SNPs in the evolution of candidate genes in *V. vinifera* L. species. Indeed, polymorphisms in the grape transcription factor family *VvMYBA* are known to be responsible for variation in the anthocyanin contents of berries of cultivated grapevine. It has also been established that white grapes arose through the mutation of two adjacent genes: a retroelement insertion in *VvMYBA1* and an SNP in *VvMYBA2* (Fournier-Level et al. 2010). The neutrality test values obtained in our study were non-significant for genes *VvMYBA1* and *VvMYBA2* in the wild grapevine samples and for gene *VvMYBA1* in the cultivated samples although the sample size may be responsible for not finding significant neutrality test. This indicates a non-departure from neutrality expectations for this gene in wild samples, which reflects equilibrium between genetic drift and neutral mutation selection (Brown et al. 2004). However, significant negative neutrality test values were recorded for gene *VvMYBA*2 in the cultivated grapevine gene pool. Such nucleotide genetic structure could be generated by linkage to a selective sweep related to the domestication of *V. vinifera* species at the locus examined, a finding that confirms the results reported by Fournier-Level et al. (2010).

The genes within each copy of the color locus are inherited together and may be considered as haplotypes. The color locus in *V. vinifera* cultivars consists mainly of HapN, HapRs, and HapB (Azuma et al. 2008; Fournier-Level et al. 2010; Kobayashi et al. 2004; Lijavetzky et al. 2006; This et al. 2007; Walker et al. 2007). HapN is presumed to be an ancestral haplotype, consisting of the functional genes *VvMYB1c* and *VvMYB2r* (Fig. 1). HapRs contains both a functional component, *VvmybA1c*, and a non-functional component, *VvmybA2w* (Fournier-Level et al. 2010), and HapB contains two non-functional components, *Vvmyb1a* and *VvmybA2w* (Walker et al. 2007). In our *sylvestris* genotypes, we detected several undescribed haplotypes in the cultivated vines and named these HapC, HapD, HapG, HapH, and HapE, although they appeared at low frequencies. The most common haplotypes in the wild group were also present in the cultivated group (HapN, HapRs, and HapF). However, haplotype distribution varied across the Mediterranean basin. Thus, western wild accessions showed a higher frequency of HapN while our eastern wild accessions showed higher frequencies of HapN and HapF. Our result support HapN as the ancestral haplotype present at both extremes of the Mediterranean basin except in the Italian wild grapevine population. This Italian population may have hybridized with recently domesticated grapevine from the eastern region. Alternatively, the low allelic richness in the Italian wild population could be the result of a genetic bottleneck effect. Garfi et al. (2013) also observed a loss of diversity in wild Italian grapevine accessions using microsatellite markers.

The Caucasian region showed the greatest diversity, with the presence of two main haplotypes N and F as the most common. The diversity of the Georgian wild compartment compared to the European natural populations was consistent with the uniqueness and originality of Georgian germplasm (*sativa* and *sylvestris*), considered according to microsatellite data the first domestication center for grapevine (Imazio et al. 2013; Myles et al. 2011). Accordingly, we tried to link our conclusions drawn at the haplotype scale with knowledge at the grape genotype scale. The pattern of genetic diversity was correlated with the traditional structure of grape agro-morphological diversity, most common use (wine *vs.* table; Levadoux 1956), geographic origin and structure assignation to eastern or western populations (Le Cunff et al. 2008). In our sample, most of the western wild grapes carrying HapN came from the Iberian Peninsula, suggesting that Spanish and Portuguese grapes have kept the ancestral haplogroup. This could either be the outcome of an isolation event affecting these varieties in a region considered a glaciation refuge zone (Olalde et al. 2002), involving limited gene flow from the east or due to their hybridization with domesticated grapevine cultivars carrying that haplotype (Arroyo-Garcia et al. 2006). The isolation of Iberian Peninsula germplasm was also proposed by Bacilieri et al. (2013) based on microsatellite data for more than 2,000 worldwide accessions. These authors identified three main genetic groups and two additional groups, which subdivided accessions according to human use (wine and table grape cultivars) and geographic origin (western regions, Balkans and eastern Europe, Caucasus and neighboring regions, Iberian Peninsula and Maghreb, Italy, and central Europe).

Further, the F haplotype was essentially found in the central European and Caucasian wild genotypes. Unlike the colored wine grapes, which appeared to randomly carry either the N or other haplotype, the near systematic presence of F haplotypes in the majority of colored table grapes supports the hypothesis of a more recent, eastern breed of the table cultivars, compared with the wine cultivars. Interestingly, these findings reinforce the idea that the western grapes have conserved the ancestral haplotypes, whereas the eastern grapes are the result of continuous and intensive breeding practices that have led to the loss of ancient diversity, even that endemic to these regions

(Levadoux 1956). This evidence supports the correlation between lower selective pressure for wine uses in the western region and an increased selection for table use in the eastern region (Arroyo-Garcia et al. 2006; Le Cunff et al. 2008; Fournier-Level et al. 2010). Moreover, the selection process for table grapes is more straightforward because of an easier process of quality trait assessment. Finally, molecular differences between the preferred presence of Rs/F haplotypes in table grape and N haplotypes in wine grape point to selective adaptation to a particular use. Thus, in contrast to the primary domestication traits, growers within each grapevine-growing region have selected distinct color variants, leading to a diversification process.

In conclusion, our results indicate that haplotype composition is a major determinant of grape skin color variation. Relationships between haplotype and total anthocyanin contents revealed two functional haplotypes (HapN/HapN) in wild accessions with higher anthocyanin levels than accessions with only a single functional haplotype. Similar results were described by Azuma et al. (2008) and suggest that the number and functional types of haplotype at the color locus are the major genetic determinants of skin color variation. The second hypothesis is that DNA polymorphisms such as SNPs (causing amino acid substitutions) in the coding region of MYB-related genes drive the differential regulation of anthocyanin biosynthesis pathway genes. An amino acid substitution may affect the promoting ability or binding of these transcription factors with the promoters of the pathway genes. Although we cannot discard that other genes could be involved in this trait. Indeed other QTLs have been identified, that also can explain part of the color diversity. For example, one of them corresponds to a mutation in the promoter region of UFGT gene (Huang et al. 2013). Then, it is necessary to work in further studies to clarify the genetic determinism of grape anthocyanin accumulation.

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Chapter 7

Pests and Diseases

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Ecological and sanitary characteristics of the Eurasian wild grapevine (*Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi) in Georgia (Caucasian region)

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Abstract

This paper shows the results of the investigation on some ecological aspects and on the sanitary status of the wild Euroasiatic native grapevine (*Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi) in Georgia (South Caucasus). This taxon is seriously endangered by human activities such as forest cleaning and setting fires. Moreover, invasive *Vitaceae* of the North American origin, imported after phylloxera (*Daktulosphaira vitifoliae* Fitch) when vineyards were being replanted, increase the risk to lose these spontaneous vines. The survey includes collection of data on the population structure, the plant sex ratio, the main botanical supporters of the vines and the associated flora, the presence of invasive vines of the North American origin and the incidence of phytophagous arthropods and pathogens. The phytosanitary study showed that monophagous eriophyid mites and exotic fungal diseases, such as downy (*Plasmopara viticola* (Berkeley and Curtis) Berlease and de Toni) and powdery mildews (*Erysiphe necator* –(Schweinitz) Burrill), cause symptoms on all the observed populations. The absence of symptoms caused by phylloxera, root-knot nematodes and root rot is remarkable. However, the level of detected injuries caused by these parasitic organisms does not seem to be a real problem for the survival of the populations.

Keywords: associated flora; downy and powdery mildew; eriophyid mites; North American *Vitaceae*; phylloxera

Introduction

The Eurasian native wild grapevine is taxonomically classified as *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi. This sub-specific taxon constitutes the dioecious ancestor of grapevine cultivars belonging to *V. vinifera* L. ssp. *sativa* (DC.) Hegi (De Candolle, 1883; Arnold, 2002). Currently, this strain is considered a threatened plant genetic resource, and it is quickly disappearing through direct and indirect human intervention (Red Book, 1982; Arnold *et al*., 1997). The causes are mainly attributed to deforestation and building activities in deforested locations. In the past, wild grapevines were used for the production of juice, wine, vinegar, tartaric acid, medicines, fishing traps and rootstocks among other things. These traditions have been carried out by

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different cultures throughout centuries, from the Hindu Kush mountain range in Asia to the Iberian Peninsula in Europe (Ocete *et al.*, 2007).

Wild vines are woody lianas that through their tendrils climb up on the nearby vegetation in order to obtain the best canopy architecture. The resulting adaptive advantage contributes to enhanced exposure to direct solar radiation and reducing competition disadvantage with other surrounding species.

Georgia is situated between the Caucasian mountain range, with some peaks over 5000 m in height, and the Black Sea. Due to its particular geographical location, the area constituted a unique refuge habitat for several plants, including *Vitis*, during Pleistocene ice ages (Ramishvili, 2001).

The main natural habitats of the wild grapevine populations are river-bank forests and some colluvial positions situated on the slopes of hills and mountains (Ramishvili, 1998; Arnold, 2002; Maghradze *et al.*, 2010), where soils are often renewed by flooding or by gravity.

Wild vines show a high foliar polymorphism. The fruiting plants produce small inflorescences with feminine flowers with reflected stamens. The male plants have bigger inflorescences constituted only by staminate flowers. Wild berries are usually small, roundish and black (Arnold, 2002). These plants have some interesting features of biotic and abiotic stress resistance that could be transferred, by selective breeding, to cultivars and rootstocks (Ocete *et al.*, 2007).

Ocete *et al.* (2011) carried out an investigation of the current state of phylloxera infestation in European countries. Data from the Caucasus region were not available at that time. Studies assaying microsatellite (short sequence repeat (SSR)), chloroplast microsatellite (cpSSR) and single nucleotide polymorphism markers on the genetic sequence of the wild grapevine from the South Caucasus region, which also includes Georgia, have stressed a high genetic drift compared with European populations (Arroyo-García *et al.*, 2006; Imazio *et al.*, 2010; Myles *et al.*, 2011). This increases the interest in the study of the main pests and diseases affecting the wild grapevine populations in this geographical area. In addition, this would contribute to the evaluation of their sanitary status after 150 years of infestation of phylloxera and American fungal diseases. Thus, the main aim of this study was to survey the sanitary status of this taxon in Georgia, with particular interest in the incidence of pests and diseases, and an evaluation of the possible competition from North American species.

Materials and methods

Field expeditions were organized to characterize the wild grapevine populations in the eastern regions of Georgia during the summer in 2008. Each population had previously been observed at the flowering time in May–June. Differentiation between *V. vinifera* wild vines and North American species was carried out by observing the main morphological discriminant descriptors. Attention was particularly focused on leaves and flowers, following the methods of Larrea (1978) and Ocete *et al.* (2006). Plant classification, including the supporting trees of the wild vines and the accompanying vegetation, was determined following the local Florae (Grossgeim, 1937–1967; Makashvili, 1991; Flora Georgia, 1971–2007) and then validated by Dr Benito Valdés from the Botanical Department of the University of Sevilla (Spain).

The observation of symptoms caused by pests and diseases was carried out on shoots, leaves and bunches of up to 3 m of canopy height. To detect any possible subterranean phytophagous and pathogens, roots were unearthed down to 40–50 cm of depth. They were

Table 1. Location of the wild populations in Georgia

Site name	Position[a]	Interval of latitude	Interval of longitude	Elevation (m.a.s.l.)
Delisi	C	41°43′27.3″–41°43′38.4″	44°42′14.3″–44°42′18.3″	648–654
Shirikhevi	A	41°57′50.5″–41°57′53.6″	44°43′0.1″–44°43′17.6″	698–707
Bagichala	C	42°2′17.3″–42°1′16.9″	44°44′17.6″–44°44′52.5″	706–718
Zhinvali reservoir	AC	42°8′39.8″	44°45′58.8″	677
Meneso	C	42°2′17.3″–42°7′24.8″	44°40′31.6″–44°46′31.5″	927
Ninotsminda	C	41°44′17″–41°44′20.8″	45°17′5.9″–45°17′8.6″	878–880
Kvetari	C	42°3′17″–42°3′38.2″	45°6′17.6″–45°6′47.6″	700–793
Sabue	C	42°2′53.4″–42°3′21.4″	45°7′8.2″–45°7′32.1″	621–649
Chachkhriala	AC	42°2′42.5″	45°9′2.4″	618
Samebis seri	AC	41°56′29.1′–41°56′32.7″	45°46′2.1″–45°46′30.6″	358–366

m.a.s.l., metres above sea level.
[a] Positions are defined as follows: A, alluvial position; C, colluvial position; AC, both alluvial and colluvial positions.

Ecological status of the Georgian wild grapevine

Table 2. Status of wild populations in their natural habitat

Populations	No. of sites	Total plants	Plants per site	Plant range
In this study	10	89	8.9	1–20
Georgia total	50	189	3.8	1–20
Italy[a]	277	1032	3.7	–
Spain[b]	378	2041	5.4	1–260

[a] Data from Biagini (2011). [b] Data from Ocete *et al.* (unpublished).

evaluated as done previously by Ocete *et al.* (2007) in the case of mite infestation and according to the OIV (2009) descriptors in the case of mildews.

Results

Ecological aspects

In this study, ten locations were surveyed (Table 1). They were considered independent populations when the distance between two sites was more than 10 km. It has to be taken into account that the male pollen grain of the studied species has a medium weight, hence it cannot be transported by wind over long distances as described by Arnold (2002). The number of vines varied between 1 and 20 plants in the different sites, with an average value of 8.9 (Table 2). The number of vines of each sex from each population is indicated in Table 3.

The data demonstrated that 18 out of 89 observed wild plants had female-type flowers (20.2%), and 24 plants had male-type flowers (24.0%). A large group of plants (48) are still unidentified, because of the short flowering period which impeded the complete field observation over the large area to survey.

The main non-vinifera grapevines were classified as American rootstock hybrids escaped from cultivation. *Vitis rupestris* and *Vitis riparia* like-to-type plants were detected in the Bagichala and Kvetari region sites. *Vitis* × *labruscana* cultivar Isabella like vines were found only in the Kvetari region.

The plant supporters and the accompanying vegetation are listed in Supplementary Table S1 (available online only at http://journals.cambridge.org).

Evaluation of pests

Data on the presence of phytophagous arthropods are reported in Table 4.

The observations carried out on roots demonstrated that, in natural habitats, no damage caused by phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Homoptera, Phylloxeridae), was found on roots and leaves. All roots showed a complete absence of symptoms caused by root-knot nematodes, such as galls and secondary rootlets (Raski, 1994). Damages caused by *Meloidogyne* were not found.

Concave felty galls situated in the lower leaf surface, which induce swellings on the lower leaf side, caused by the erineum strain of *Colomerus vitis* (Pagenstecher) (Acari, Eriophyidae), were observed on all the populations studied in the present survey. Symptoms were very frequent, affecting almost all of the vines, but damages caused by this pest were not serious (levels 1–3), and did not affect the viability of the liana.

Another mite found on the leaves of most of the prospected populations was the grape rust mite, *Calepitrimerus vitis* (Nalepa) (Acari, Eriophyidae). Its distribution and level of infestation, usually scored at level 1, was lower compared with *C. vitis*.

Evaluation of diseases

Data on the presence of symptoms caused by diseases are shown in Table 4. Symptoms of root rot were absent on all samples.

On the parts of the plants above ground level, symptoms of infection were caused by North American fungal species which included powdery and downy mildews, *Erysiphe necator* (Schweinitz) Burrill and *Plasmopara viticola* (Berkeley and Curtis) Berlease and de Toni, respectively.

Symptoms of powdery mildew on wild vines were found on leaves, shoots (Chleistotecia) and, far more rarely, on the bunch. These symptoms affected virtually all the populations studied in Georgia. The degree of intensity of the infection on wild vines was rated between 1 and 3 through observing the leaves according to the descriptors. This corresponds to a low infection.

Table 3. Sex of flowers of wild vines

Site name	No. of plants	Female	Male	Not yet identified
Delisi	7	1	3	3
Shirikhevi	8	3	5	0
Bagichala	18	4	9	5
Zhinvali reservoir	4	–	1	3
Meneso	2	2	–	0
Ninotsminda	12	2	3	8
Kvetari	20	2	2	16
Sabue	8	3	–	5
Chachkhriala	1	1	–	–
Samebis seri	9	–	1	8
Total	89	18	24	48
%	100	20.2	27.0	53.9

Table 4. Number of affected plants and evaluation of infestation/infection by parasites[a]

Site name	No. of plants	*Colomerus vitis*	*Calepitrimerus vitis*	*Erysiphe necator*	*Plasmopara viticola*
Delisi	7	7 (1–3)	3 (1)	5 (1–3)	2 (1)
Shirikhevi	8	8 (1–3)	3 (1)	4 (1–3)	1 (1)
Bagichala	18	18 (1–3)	5 (1)	11 (1–3)	3 (1–3)
Zhinvali reservoir	4	4 (1–3)	1 (1)	3 (1–3)	0
Meneso	2	2 (1)	0	2 (1–3)	0
Ninotsminda	12	12 (1–3)	3 (1)	7 (1–3)	3 (1–3)
Kvetari	20	20 (1–3)	7 (1–3)	11 (1–3)	4 (1)
Sabue	8	8 (1–3)	3 (1–3)	3 (1–3)	2 (1)
Chachkhriala	1	1 (1)	0	0	0
Samebis seri	9	9 (1–3)	2 (1)	3 (1)	1 (1)
Total	89	89	27	49	16
%	100	100	30.3	55	17.9

[a] For each species, the number of affected plants and level of infestation (in parentheses) are indicated, following the scale of Ocete *et al.* (2007) for mite infestation, and the OIV (2009) descriptors for mildews. In the case of mites, the evaluation situated between 1 and 3 means that the mite affected 10–25% of the leaves.

Typical symptoms of downy mildew were found in Georgian wild vines on leaves (similar to oil spots) and shoots longer than 10 cm; this also occurs in the case of cultivars. Damage on bunches was less frequent. Finally, symptoms caused by this fungal species were less frequent than those caused by powdery mildew, as indicated by the degree of infection rated as 1 on average.

Discussion

The evaluation of the status of wild populations in this survey demonstrated a higher density of plants per site (8.9) compared with data from the whole of Georgia (3.8 plants per site) (Maghradze *et al.*, 2011), Italy (3.7) and Spain (5.7) (Table 2). In both Western European countries, the number of populations is higher than in Georgia and, as a consequence, a higher number of vines have been identified. This is probably also due to the fact that in the Italian and Spanish surveys, several natural reserves have been involved, whereas the investigation in Georgia was done outside the boundaries of protected areas.

Usually, the number of males identified in each population was higher than the number of females. In the sites of Zhinvali and Sabue, there are no female plants. This makes seed reproduction impossible. In general, populations are very small so their short- and medium-term viability is expected to be very low (Table 3).

It is necessary to underline that in the Caucasian region, where Vavilov (1926) found the highest diversity of vines in the cradle of viticulture, there were 55 productive female cultivated varieties (13.3% of total germplasm; Ampelography, 1970; Maghradze *et al.*, 2010). Among these, the two cultivars 'Asuretuli Shavi' and 'Tavkveri' are included in the official list of cultivated varieties of Georgia (Law, 1998) and are spread throughout the Kartli province of East Georgia. The female cultivars can be pointed as a relict step in the history of Georgian viticulture: they show the passage from the domestication of wild vines to the cultivation of selected hermaphrodite varieties during an early development phase of this crop.

The accompanying vegetation of the vines is the characteristic flora of the Caucasian natural areas under 1000 m of altitude with low human impact, where several species of fruit trees took refuge during the Quaternary ice age. This flora is typical of temperate forests in the Palaearctic ecozone, where there is great biodiversity due to the confluence of Central European, Central Asian and Middle Eastern botanical provinces. This alluvial formation with deciduous species constitutes the Euxine–Colchic forest of the South Caucasus, which stretches eastward towards the shores of the Caspian Sea, where the Tertiary botanical species took refuge (Moore, 1982).

Supported trees and bushes with thorns (such as *Crataegus* ssp. and *Prunus* ssp.) are supposed to have played an important role in the protection of wild vines from wild or domestic animals.

As in Europe, some of the American rootstock hybrids and *Vitis* × *labruscana* are gradually colonizing the river banks and slopes of the hills in Georgia, taking over the niche of autochthonous wild vines (Cholokashvili, 1983), as it was observed in several European populations (Arrigo and Arnold, 2007). Such is the situation in the Bagichala and Kvetari sites. Such plants are more invasive and show a higher resistance to North American imported mildews compared with native wild vines: this is why they are involved in the extinction of wild autochthonous grapevines in

the Valencia region (Spain; Laguna, 2003a, b), Têt river valley (France), Montseny Reserve of the Biosphere (Spain), and in several parts of the Rhone, Rhine, Danube, Ebro, Guadalquivir, Duero and other important European rivers and their tributaries (Terpó, 1969, 1974; Ocete *et al.*, 2007). This is another reason for the urgent need of protection of the biodiversity of the Georgian wild grapevine, which is the earliest known domestication centre of the vine. In the Eastern part of the Iberian Peninsula, it was one of the main causes for wild grapevine disappearance (Laguna, 2003a, b).

The absence of phylloxera infestation is due to the temporary flooding of soil profiles in all of the European locations. These lianas grow in sites where edaphic conditions such as permanent or temporary anoxic conditions caused by flooding make them unsuitable for the development of phylloxera. Meanwhile, laboratory experiments with artificial infestation indicated that Eurasian wild grapevines exhibited nodosities and tuberosities on roots caused by a homopteran under induced artificial infestation in pots (Ocete *et al.*, 2011).

In spite of considerations on the infestation of roots of wild grapevines found in France by Camille St. Pierre and included in De La Branchere (1876), phylloxera had little direct impact on the remaining wild vines (Ocete *et al.*, 2006).

Around Georgia, a homopteran was detected in Southern Russia in 1863 (Negrul, 1952). In 1881, it was cited in the West Georgian province of Abkhazeti, close to the city of Sokhumi located on the Black Sea coast. Between 1889 and 1891, the vineyards situated in Western Georgia were infested. In Eastern Georgia, the pest was found in Tbilisi in 1884 (but it was immediately eliminated) and in the province of Kartli in 1893. Later, between 1906 and 1910, the symptoms were found in the province of Kakheti (Kantaria and Ramishvili, 1983; Ramishvili, 2001). The damage throughout the country was extensive, and losses were so high that viticulture and winemaking ceased to be prosperous activities. The vineyard acreage was dramatically reduced. To control phylloxera and other diseases, the 'Caucasus Phylloxera Committee' was established in 1880, playing an important role in the detection of infested vineyards, introducing innovative methods to fight against parasites and describing local varieties under the threat of extinction. The method of grafting local varieties on American rootstocks was introduced in the last decade of the 19th century, playing a very important role in saving Georgian viticulture and winemaking (Lomineishvili and Gaprindashvili, 1990).

The absence of damage caused by nematodes is probably due to the action of water contained in the profile of the soil mentioned previously, according to Palm and Walter (1991).

The erineum strain of *C. vitis* caused symptoms on 100% of the studied vines, with a low (1–3) intensity of attack, as occurred in the case of the Spanish and French populations (Lara and Ocete, 1992; Ocete *et al.*, 2007, 2008). It is a monophagous species (Arnaud and Arnaud, 1931), widely distributed in the vineyards of both hemispheres (Keifer *et al.*, 1982; Dennil, 1986). This mite has two strains which are more commonly found on cultivars: the bud strain and the leaf curl strain (Reyes, 2004). Through the genetic research approach and characterization, the two strains would belong to distinct species (Carew *et al.*, 2004). In those nests, several natural enemies of the erineum strain mite can be found, mainly *Phytoseiidae*, *Tydeidae* and *Cecidomyida*, and all of them constitute a new target to be investigated in the future. Some predatory species belonging to the cited families cannot be found in vineyards due to the use of pesticides (Ferragut *et al.*, 2008).

C. vitis is another monophagous species detected in 62% of the European populations sampled between Portugal and Hungary (Ocete *et al.*, unpublished data). It caused a low level of infestation on 30.3% of the vines found in Georgia belonging to eight populations observed, with an overall lower level of infestation compared with other mite species. After bud burst (D phenophase according to Baggiolini (1952)), females that have broken the diapausa begin to feed, resulting in small spots that can be seen against light. Symptoms caused by this phytophagous were found in different vineyards of Europe, America, South Africa and Australia. Usually, it is considered as a secondary pest (Sazo Rodríguez *et al.*, 2003). Injuries caused by a high infestation of both cited mites on Australian vineyards were referred to as restricted spring growth (Bernard *et al.*, 2005).

Because of the impossibility of long migrations of the two obligatory monophagous parasitic eriophyids and their wide presence on the majority of wild grapevines, we can assume that these mites have always coexisted with their primary host since ever and were transferred to cultivars during the domestication processes.

Several support trees, belonging mainly to the *Populus* and *Quercus* genera, are infected by *Armillaria mellea* (Vahl: Fr.) Kummer in Georgia. This fungal disease caused the hyphae to produce abundant white mats between the hardwood and the bark, but it is absent in the roots of the vines. It is an interesting fact when focusing on the possibility of getting new rootstocks using wild vines in breeding programmes.

Powdery mildew symptoms were detected in 55% of the studied vines. This percentage is similar to Southern Spanish populations, according to data from Ocete *et al.* (2007). Its level of infestation varied between low and

medium. Only one population was free from the disease, perhaps due to the fact that it contained only one vine.

In the Old World, the first damages caused by powdery mildew were discovered in England by Berkeley (1847), and were later detected on cultivars situated in France (Cortés, 1854; Müller, 1882). Eight years later, the fungal disease had invaded the vineyards of Europe, Northern Africa and Asia Minor (Le Canu, 1862). In Georgia, this disease was discovered in the middle of the 19th century (1857 in Guria province) in Western Georgia: it destroyed high vineyards in the provinces of Guria and Samegrelo (Kantaria and Ramishvili, 1983; Ramishvili, 2001). Nowadays, the pathogen can be found in all wine-producing areas, under dry weather conditions and average temperatures between 15 and 35°C (Pérez de Obanos, 1992; Pearson and Goheen, 1996).

On the other hand, downy mildew affected 18% of the vines, usually with a lower intensity than *E. necator*. No symptoms were found in the three populations with a lower number of vines. In the case of Southern Spanish populations, this disease affected about 60% of the 200 populations studied by Ocete *et al.* (2007).

Downy mildew was detected in the south of Western France, near Bordeaux in about 1878 (Millardet, 1885). Four years later, the disease affected all French vineyards and adjacent countries (Urien de Vera and Diego-Madrazo, 1891) and reached South Caucasus in the last two decades of the 19th century (Kantaria and Ramishvili, 1983; Ramishvili, 2001).

The presence of both North American mildews on Georgian populations and in the rest of the Eurasian wild grapevines studied in Europe is a legacy from cultivars infected by the massive importation of American vines occurred in the 19th century. All cultivars of *V. vinifera* are susceptible to this fungus. Only the North American species, mainly *V. rupestris* and *Vitis rotundifolia*, exhibited an important level of resistance because they had evolved with this pathogen (Leroux and Clerjeau, 1985). The easy transportation of the spores from vineyards to natural habitats induces the infections of wild vine populations situated in remote sites.

This phytosanitary study demonstrates that, today, parasitic organisms are not the main problem for the survival of relic populations. This is a very important conclusion, as it was widely believed that phylloxera was one of the main causes of the reduction of wild grapevine populations in Georgia. Despite this fact, the fungal diseases probably had a heavy impact on wild grapevine individuals, leading to the death of many plants growing in sites which are more suitable for the development of these pathogens. The remaining wild plants could be descendants of those that exhibited a higher tolerance or were situated in habitats under conditions which were not suitable for heavy mildew infection.

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Sanitary status of the Eurasian wild grapevine in the South Caucasian region

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Summary

A prospecting on the sanitary status of the aerial organs and roots of the Eurasian wild grapevine, *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi, was carried out on 14 natural populations situated along river bank forests, floodplains and colluvial positions in Georgia (Marneuli, Mtskheta and Gori districts, Gardabani Protected area and Lagodekhi Reserve), Armenia (Akhtala and Tavoush regions) and Azerbaijan (Quba region). These zones are included within the Holarctic kingdom, Eurosiberian region, and to the Caucasian, Euxine and Hyrcanian biogeographical provinces. The results of study indicate that roots are free of symptoms caused by phylloxera, rot fungi and root-knot nematodes. Symptoms caused by the erineum strain of *Colomerus vitis* (Pagenstecher) and *Calepitrimerus vitis* (Nalepa) (Acari, Eriophyidae) are frequent. On the other hand, damages caused by powdery and downy mildews, *Erysiphe necator* (Schweinitz) Burrill and *Plasmopara viticola* (Berkeley and Curtis) Berlease and de Toni), respectively, show an irregular intensity on leaves belonging to different vines from each location.

K e y w o r d s : *Vitis sylvestris*; mites; nematods; phylloxera; Oidium; Mildew.

Introduction

Vitis vinifera L. ssp. s*ylvestris* (Gmelin) Hegi constitutes the only taxon of the cited genus growing in natural ecosystems of Eurasia from Afghanistan to the Iberian peninsula (Arnold 2002) and the African Maghreb (Ocete *et al.* 2007). Fossils of grapevine from Upper Pliocene were found in the territory of Azerbaijan (Negrul 1959). The South Caucasian region constituted a refuge for this dioecious parental of grapevine during ice ages of the Pleistocene (Musayev and Akparov 2013).

The first confirmation of grape domestication is evident in the Shulaveri – Shomu Tepe culture (Georgia and Azerbaijan) archaeological findings, where wine vessels and seeds from cultivated grape, from around 8,000 B.P. were discovered (Chilashvili 2004). This process of human selection developed almost 800-900 cultivars existing in the South Caucasian area (Negrul 1970), considered the region to be the main cradle of viticulture and winemaking (Vavilov 1926). Wild grapes still constitute a resource for countryside people living in the region to produce medicines, wine, including a flavored dessert one adding aromatic male inflorescences at flowering time (Babayev 1988, Oganesyan 2005, Rivera *et al*. 2012). These inflorescences are also used for artificial pollination of functionally female cultivars (Efendiyev 1972, Cholokashvili 1983). The flowers are good honey organs (Cholokashvili 1983) and their boiled mixture has been suggested as a method to preserve the wine by Alishan (1877). The unripe fruits are used for preparation of a marinade (Sosnowski 1947, Cholokashvili 1983) or for a special sauce (Pruidze 1974).

The coexistence of such plant material with pests and diseases for years could be a source of environmental adaptation. So the aim of the present paper was to study the sanitary status of wild grape populations situated in alluvial and colluvial positions in Armenia, Azerbaijan and Georgia with the idea to evaluate the current situation for its protection in the South Caucasus.

Material and Methods

The sanitary prospection of natural populations of wild grape was organized in Georgia, Armenia and Azerbaijan in October 2013. These zones are included within the Holarctic kingdom, Eurosiberian region, and to the Caucasian, Euxine and Hyrcanian biogeoghraphical provinces. The location based on GPS coordinates and the habitats of the different populations studied is shown in Tab. 1. To detect the presence of symptoms caused by parasitic organisms on roots, they were unearthed up to 40 cm of depth - minimum one plant per population was observed. Samples of fine roots were observed under binocular in order to monitor damages caused by phylloxera, *Daktulosphaira vitifoliae* (Fitch) (Homoptera, Phylloxeridae), root-knot nematodes and rot fungal diseases (Tab. 2). In the aerial part of the all inspected vines, samples of 30 leaves per plant were observed from the available shoots up to 4 m height to examine symptoms caused by parasitic species**.**

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Table 1

Location of studded *Vitis sylvestris* populations in Georgia, Azerbaijan and Armenia in 2013

Site name	District	River basin	Interval of latitude	Interval of longitude	Position*
Georgia					
Nakhiduri	Marneuli	Ktsia	41º29′26,5″ - 44º40′ 51″	41º29′13,1″ - 44º41′22,6″	C
Gardabani protected area	Gardabani	Mtkvari	41º22′19″ - 45º4′6,3″	45º4′37,8″ - 45º4′37,8″	Flood plain
Tsitsamuri	Mtskheta	Aragvi	41º52′28″ - 44º43′51,2″	41º52′38,3″ - 44º43′ 57,3″	C
Tedotsminda	Gori	Liakhvi	42º2′4,1″ - 44º3′42,1″	42º2′20,7″ - 44º3′19,4″	C
Skra	Gori	Mtkvari	41º59′11,7″ - 44º2′47,7″	41º59′13,5″ - 44º2′47,3″	C
60s quarter of Lagodekhi presrv	Lagodekhi	Matmiskhevi	41º48′2,7″ - 46º19′12,2″	41º48′45″ - 46º20′24,8″	A
Azerbaijan					
Guruchai-1	Quba	Guruchai	41º24′1,3″	48º26′37,6″	Flood plain
Guruchai-2	Quba	Guruchai	41º26′3,3″ - 48º 33′50,6″	41º26′3,8″ - 48º33′ 41″	Flood plain
Qusarchai- 1 & 2 (Rostov road)	Quba	Qusarchai	41º28′6,3″ - 48º 33′ 59,9″	41º28′9,8″ - 48º33′ 57″	Flood plain
Dellekkend	Quba	Guruchai	41º24′37,8″	48º35′ 13″	Flood plain
Ağbil	Quba	Qusarchai	41º25′32″ - 48º34′4,7″	41º25′35,4″ - 48º33′54″	Flood plain
Armenia					
Akhtala	Akhtala	Debed	41º6′18,3″ - 44º42′23	41º7′15,8″ - 44º45′16,3″	C
Getahovit	Tavush	Getik	40º54′6″ - 45º7′53″	40º54′ 8,7″ - 45º7′ 9,6″	C

* A means alluvial position (riverbank forest); C: colluvial position (slop of a hill).

Table 2

Number and percentage of affected plants (2013)

Site name	N. plants	*Colomerus vitis*	*Calepitrimerus vitis*	*Erysiphe necator*	*Plasmopara viticola*	Phylloxera	Nematodes	Root rot
Skra	4	4 a	0 a	1 a	2 a	0	0	0
Tsitsamuri	7	6 a	2 a	1 a	7 a	0	0	0
Lagodekhi	9	8 a	0 a	0 a	0 a	0	0	0
Nakhiduri	11	1 c	0 a	0 a	1 a	0	0	0
Gardabani	12	8 a	10 c	5 a	10 a	0	0	0
Tedotsminda	19	16 a	0 a	6 a	19 b	0	0	0
Total GEO	62	43 A (69.4 %)	12 A (19.4 %)	13 A (21.0 %)	39 A (62 9 %)	0	0	0
Guruchai-1	4	4 a	4 a	0 a	4 a	0	0	0
Qusarchai-2	4	4 a	4 a	4 b	4 a	0	0	0
Dellekkend	5	5 a	3 a	4 b	5 a	0	0	0
Ağbil	8	8 a	7 a	8 c	8 a	0	0	0
Guruchai-2	11	11 a	9 a	1 a	11 a	0	0	0
Qusarchai-1	11	6 a	10 a	3 a	11 a	0	0	0
Total AZE	43	38 AB (88.4 %)	37 B (86.0 %)	20 B (46.5 %)	43 B (100 %)	0	0	0
Akhtala	16	14 a	10 a	11 a	16 a	0	0	0
Getahovit	8	7 a	6 a	4 a	7 a	0	0	0
Total ARM	24	21 A (87.5 %)	16 B (66.7 %)	15 B (62.5 %)	23 B (95.8 %)	0	0	0
Total all	129	102 (79.1 %)	65 (50.4 %)	48 (37.2 %)	105 (81.4 %)	0	0	0

Note: Small letters (a, b, c) show differences between country regions. Capital letters (A, B) show differences between countries. When $p < 0.05$ interaction was considered as statistically significant.

Statistical analyses: Chi-square test was used to compare the categorical data within and between groups in order to discern effects of pathogen infection and between populations inside the countries or average for the countries studied. Fisher's exact test was applied where the expected values were less than 5 in a 2 x 2 table.

Results and Discussion

No symptoms caused by phylloxera, root-knot nematodes and fungi were found on roots (Tab. 2). It has to be remarked that Eurasian wild grape has no tolerance to the root phase of this homopteran under artificial infestation in the laboratory tests. So, the absence of the insect in these habitats sampled seems due to the flooding of the soils several months each year (Ocete *et al*., 2011). This edaphic condition could also be responsible for the absence of damages caused by nematode species of *Meloidogyne* and root rot fungal species of *Armillaria.*

The presence of the erineum strain of the mite *Colomerus vitis* (Pagenstecher) (Acari, Eryophidae) is evident on the majority of all the populations observed (Tab. 2), as it was related before on another Georgian population

(Ocete *et al*. 2012). In the case of the present study, the occurrence of this mite was registered in 79.1 % of the vines. Its level of infestation shows small differences along the different South Caucasian countries. Infestation caused by *Calepitrimerus vitis* (Nalepa) (Acari, Eryophidae) affected half the number of observed wild vines (50,4 %). Its percentages of infestation varied from 19,4 % in Georgia, 66,7 % in Armenia to 86 % in Azerbaijan. Powdery mildew, *Erysiphe necator* (Schweinitz), and downy mildew, *Plasmopara viticola* (Berkely & Curtis) Berlease & de Toni were observed in 37,1 % and 81,4 % of the vines, respectively – so this study demonstrated that downy mildew is more frequently found than powdery mildew on South Caucasian wild grape. *P. viticola* is more widespread for Armenia and Azerbaijan, and *E. necator* for Azerbaijan. However, the presence of both monophagous eryophid mites could indicate that they were transferred to cultivars along the domestication process. On the contrary, mildews were imported from North American grapevine species and were transferred from vineyards to the wild habitats.

Conclusions

A prospecting on the sanitary status of the aerial organs and roots of the Eurasian wild grape, *Vitis vinifera* s*ylvestris*, was carried out on 14 natural populations situated along river bank forests, floodplains and colluvial positions in Georgia, Armenia and Azerbaijan. The results indicate that roots are free of symptoms caused by phylloxera, rot fungi and root-knot nematodes. Symptoms caused by the erineum strain of *Colomerus vitis* (Pagenstecher) and *Calepitrimerus vitis* (Nalepa) (Acari, Eriophyidae) are frequent. On the other hand, damages caused by powdery and downy mildews, *Erysiphe necator* (Schweinitz) Burrill and *Plasmopara viticola* (Berkeley and Curtis) Berlease and de Toni), respectively, show an irregular intensity on leaves belonging to different vines from each location. In case of fungal diseases favorable climatic conditions (in majority) plus some interaction of genotypes (for single genotypes) can be considered due to low general resistance of *V. vinifera* towards the fungal deceases. However, the absence of symptoms caused by Phylloxera, nematodes and root-rot fungi could be due to edaphic conditions, not to a real tolerance/resitance of the vines. This fact is important to take into account for the *ex situ* conservation of this taxon.

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El patrimonio viticola de Georgia: el estado sanitariode sus poblaciones silvestres

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RESUMEN

En el presente trabajo se muestra a este pais como una de las cunas de la viticultura. Se recogen las principales variedades de cultivo autóctonas de Georgia, tanto hermafroditas como femeninas. Al mismo tiempo, se hace una caracterización de 10 poblaciones silvestres, indicando las principales artrópodos fitófagos y enfermedades fungicas que las afectan. Finalmente, se comenta la existencia de posibles vitaceas exóticas invasoras, importadas tras la infestación filoxerica.

Palabras clave: artrópodos fitófagos, biodiversidad,enfermedades fungicas, Iberia*, Vitis vinifera sylvestris*.

ABSTRACT

The present paper shows Georgia as a cradle of the viticulture. The main native hermafrodite and female cultivars are refereed. A characterization of 10 wild grapevine populations was also carried out, paying a special attention to phytophagous artrhopods and fungal diseases affecting them. On the other hand, the presence of possible invading exotic vitaceae, imported due to filoxerainfestation, is indicated.

Key words: biodiversity, fungal diseases, phytophagous arthropods, Iberia, *Vitis vinifera sylvestris.*

INTRODUCCIÓN

El estado asiatico de Georgia, la antigua Iberia, tiene una extensión de 69.700 Km^2. Al norte limita con Rusia y al sur con Turquia, al sudeste con Armenia y al este con Azerbaijan. Se extiende desde la cordillera del Caucaso hasta las costas del mar Negro. Dichas montafias, que llegan a superar los 5.000 m de altitud, sirvieron como muro de defensa frente a los hielos del Pleistoceno. Por esa razón, multitud de especies botanicas, varias de ellas frutales, se refugiaron en ese

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territorio y, posteriormente, fueron paulatinamente domesticados por el hombre, como es el caso de la vid (De Candolle, 1883).

En Georgia existen macrofósiles de vitaceas, tallos, hojas y semillas provenientes de facies terrestres del Mioceno en la región de Akhaltsikhe (Chiaureli, 1989).

El equipo de Costantini et al. 2006 y Rusishvili (2007) investigaron todos lo restos arqueológicos de vid desde el Neolitico, tanto semillas, como polen y restos de madera. Lo que prueba la presencia de este cultivo en Georgia desde hace varios milenios, concretamente entre el VI y IV a.C., segun la cronologia de los centros de domesticación de la vid realizada por Forni (2005-2006). La presencia de vid silvestre en esta zona caucasica fue referida ya en el s. XIX por Kolenati (1846).

En la zona meridional del Caucaso existe una gran biodiversidad de la vid, tanto silvestre como de cultivo, por lo que Vavilov (1926) la integra en el denominado *Triangulo de la uva fertil*. Por ello se la considera como la cuna de la Viticultura, ya desde la mas remota antigiiedad. Ya, mas de medio siglo antes, Hehn (1870) indicaba que las regiones situadas entre el Caucaso, Monte Ararat y Cordillera de Taurus eran la cuna de la viticultura antigua. Scienza (2004) sefiala la presencia de uvas con caracteristicas intermedias entre silvestres y cultivadas en la franja boscosa situada entre el Caucaso y Kachetia. Hasta el momento, los restos de vino mas antiguos que se conocen proceden de recipientes ceramicos del yacimiento neolitico de Shulaveris Gora, datables hacia 6.000 a.C. (Mc Govern, 1999) (Figura 1).

La Biblia, que se hace eco de muchas tradiciones anteriores, sefiala al monte Ararat (Armenia), situado casi en el *Triangulo de Vavilov,* como el punto donde encalló el Arca de Noe y le atribuye la plantación de la vifia y el uso del vino. Asi, en el Genesis (IX, 20-21) se lee: *Noe, que era labrador comenzó a labrar la tierra y plantó una vifia. Y bebiendo de su vino quedó embriagado.*

Desde muy antiguo, el ser humano fijó su interes por la vid, ya que de ella se obtienen bayas para su consumo en fresco o pasificadas, savia, agraz, mosto, vino, vinagre, que ha sido un importante conservante alimentario junto a la sal. Loscitados fluidos cuentan con varias aplicaciones medicinales conocidas desde hace milenios. Y, ademas, su madera, aparte de servir de combustible, fue aprovechable por ser muy resistente a la intemperie para la fabricación de puertas y ventanas (Ocete *et al*., 2007).

Por otra parte, debe tenerse en cuenta que el mosto cuenta con un cierto porcentaje de acidos organicos. De todos ellos, el acido tartarico, 2,3 dihidroxibutanodioico, un subproducto de la elaboración de vinos que se sigue reciclando en la actualidad. Este, aparte de tener usos enológicos y de conservante de otros alimentos, ha sido un importante coadyuvante en la alfareria, para mejorar las pastas ceramicas (Carrefio, 2005). De hecho, hoy en dia, suele ser un componente frecuente de los cementos odontológicos.

Las poblaciones de vid silvestre son eminentemente dioicas. Durante el Neolitico se pasó de la recolección de la vid de las parras femeninas situadas, fundamentalmente, en los bosques de ribera o coluviales, a su cultivo. El hombre se dio cuenta que existian algunas cuantas plantas que podian dar fruto directamente; es decir, eran hermafroditas, autofecundables, formadas por mutación en el seno de las poblaciones silvestres. Por lo tanto, la domesticación de la vid se debe entender como un proceso evolutivo regulado por el hombre, cuyos objetivos pragmaticos mas destacables han sido las de fijar determinados caracteres: hermafroditismo, tamafio de la baya y del racimo, contenido de azucar y acidos del mosto, constancia del nivel de producción, etc. Hay que resaltar, ademas, la tolerancia a las condiciones medioambientales (Ocete *et al.,* 2007). Tengase en cuenta que las parras proceden de suelos encharcados, ya que las vides silvestres son hidrófilas, y se tienden a plantar en terrenos mucho mas secos, donde predominan ecotipos mesofiticos e, incluso, xerofiticos.

En el transcurso del proceso de domesticación fueron diferenciandose las tres proles descritas por Negrul (1938), que estuvo trabajando una epoca en la Estación de Viticultura y Enologia de Telavi: *occidentalis*, a las que pertenecen las viniferas con bayas pequefias cultivadas en Europa central y occidental; *orientalis*, con grandes racimos consumidos, generalmente, como uva de mesa en oriente y *póntica*., con caracteristicas intermedias entre las dos anteriores, cultivadas en la cuenca del Mar Negro. Esta ultima se haya integrada por viduefios propios de Asia Menor y Europa oriental (Figura 2).

El cultivo de la vid en Georgia viene ya referido por Estrabón (s. I). De acuerdo con la tradición georgiana, en el s. IV d.C., Santa Nino, una nifia de la Capadocia fue el vector que introdujo el cristianismo en la región. Se la representa siempre con una cruz de madera de vid, atada con sus propios cabellos. En la ornamentación de muchas iglesias aparecen relieves de vides y, por todo el pais, se encuentran bodegas antiguas (*marani*) con sus lagares excavados en grandes troncos de olmo y sus tinajas de vino enterradas, para que conservasen una temperatura estable y adecuada (Figura 3).

Durante la etapa de pertenencia de Georgia a la URSS llego a tener unas

140.000 ha dedicadas al vifiedo, en la actualidad este ha quedado reducido a unas

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37.000 ha (Agro Census, 2004), dados los conflictos con Rusia, que era el principal importador de sus vinos y destilados. La región de Khakhetia, situada al este del pais, produce casi el 70% del volumen de vino nacional (Gagunashvili, 2006). El numero de variedades de cultivo asciende a 525, segun Ketskhoveli *et al*. (1960).

El objetivo del presente articulo es mostrar el importante patrimonio varietal del pais, tanto cultivado como silvestre, asi como describir el estado sanitario de este ultimo.

MATERIAL Y METODOS

La información sobre vid cultivada procede de los archivos del Instituto de Horticultura, Viticultura y Enologia (IHVO) y la correspondiente a las silvestres ala prospección realizada durante el verano de 2008 en 10 poblaciones del este del pais, cuyas coordenadas aparecen en la Tabla 3. La identificación de los tutores y flora adyacente se ha realizado mediante claves botanicas (Grossgeim, 1942 y Makashvili, 1991, con la ayuda del Dr. Benito Valdes, del Departamento de Botanica de la Facultad de Biologia de Sevilla.

Para el estudio de los parasitos de la vid silvestre, se han observado tanto los órganos aereos como las raicillas, hasta una profundidad maxima de unos 40 cm.

Por otro lado, tanto en la zona de las poblaciones resefiadas como en la costa de Mar Negro, se ha puesto un especial empefio en la identificación de vitaceas exóticas invasoras que pudieran constituir un peligro potencial para el desplazamiento de las parras silvestres de sus habitats naturales.

RESULTADOS Y DISCUSION

La lista de variedades recomendadas incluye 37 viniferas y 14 de uva de mesa (Tabla 1). De las primeras, 31 son autóctonas y 6 introducidas (Ley de 1998).

Tabla 1. VARIEDADES RECOMENDADAS EN GEORGIA

Hollejo	Viníferas	Uva de mesa
blanco	Avasirkhva *	Gorula **
	Chinuri **	Kartuli Saadreo
	Goruli Mtsvane * - **	Kolkhuri
	Kapistoni *	Muskaturi Rkatsiteli
	Kisi **	Sakhalkho Tetri
	Khikhvi **	Tbilisuri
	Krakhuna *	Karaburnu
	Mtsvane Kakhuri **	Khalil
	Mtsvane Kakhuri-Clone 12 **	Chasselas blanc
	Rkatsiteli **	Tabriz (sin. Ganjuri)
	Rkatsiteli – Clone 48 **	
	Tsitska *	
	Tsolikouri *	
	Tsulukidzis Tetra *	
	Aligote	
	Shardone	
	Pinot blanc	
coloreado	Aladasturi *	Budeshuri Tsiteli **
	Alexandrouli *	Tskhenis Dzudzu
	Chkhaveri *	Tskhenis Dzudzu from Abkhazia
	Katchitchi *	Muscat d'Alexandrie

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	Mujuretuli *
	Ojaleshi *
	Orbeluri Ojaleshi *
	Otskhanuri Sapere *
	Saperavi **
	Saperavi – Clone 359 **
	Saperavi Budeshurisebri **
	Shavkapito**
	Tavkveri **
	Usakhelouri *
	Cabernet Sauvignion
	Malbek
	Merlot
	Pinot noir

*Variedades recomendadas para Georgia occidental; **Variedades recomendadas para Georgia oriental;

*** Variedades recomendadas en ambas regiones.

La materia prima para la producción de vino son las variedadesautóctonas, en el 94% de las vinificaciones (Agro Census, 2004), aunque ahora se usan tambien castas internacionales, como Cabernet Souvignon, Pinot noir, Pinot blanc, Aligote, Shardone y otras (Figura 4).

El numero de variedades femeninas ocupa el 13.3% del germoplasma viticola (Ampelography of the Soviet Union, 1946-1970), donde de los 414 viduefios descritos, 55 tienen flor femenina (Tabla 2). Este hecho apunta a la domesticación directa de ejemplares productivos silvestres de la zona. Las tecnicas quimiotaxonómicas basadas en el empleo de microsatelites de ADN confirman el estrecho parentesco de los viduefios cultivados con los ejemplares silvestres (Vouillamoz *et al*., 2004).

Tabla 2. VARIEDADES FEMENINAS DE CULTIVO

1	Abatsvizh	Chkhushi	Livanuri Tetri
2	Abkhazuri	Chitistvala Adzharuli	Mauri Tetri
3	Abkhazura Shavi	Dudghushi	Mkhargrdzeli
4	Adreuli Tetri	Dziganidze	Mskviltvala Rachuli
5	Akomshtali	Dzveli Alexandrouli	Mtsvivani Rachuli
6	Amlakhu	Jineshi	Muradouli
7	Amokhpizh	Jvari	Opoura
8	Apapnizh	Kakhis Tetri	Rko Shavi
9	Asuretuli Shavi	Kapistoni Gaghmouri	Rko Tetri
10	Atsisizh	Kapistoni/Kabistoni Tetri	Sapena
11	Atskhouzh	Kharistvala Meskhuri	Shavshuri
12	Atslizh	Kharistvala Tetri	Shonuri
13	Atsvimkha	Khoteura	Tavkveri
14	Avshiluri	Khunalizh	Tchetchibera
15	Azhapsh	Korkaula	Tchodi
16	Azhkapsh	Krakhuna Shavi	Tkis Kurdzeni
17	Batomura	Ktsia Tsiteli	Tsvrimala
18	Bazaleturi	Kuprashviliseuli	
19	Brola	Lakoiazh	

Las variedades femeninas Tavkveri y Asuretuli Shavi siguen incluidas enel listado oficial (Ley de 1998).

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Respecto a las variedades hermafroditas, Rkatsiteli y Saperavi constituyen los buques insignia de la viticultura georgiana (Galet, 1990). Rkatsiteli se emplea para la producción de vino blanco en diferentes tipos de suelo y climas. Presenta resistencia a la helada y tiene cierta tolerancia a filoxera y oidio, por lo que es la variedad mas empleada. Saperavi es una uva tinta con pulpa coloreada, ideal para la producción de vinos tintos de calidad. Se adapta muy bien a diferentes perfiles edaficos y climas. Constituye una de las castas mas resistentes a las heladas, con mediana tolerancia a las especies fungicas norteamericanas (Beridze, 1965, Ramishvili, 1986).

La localización de las poblaciones silvestres muestreadas aparecen en la Tabla 3. La letra A significa que la población tiene una posición aluvial, mientras que las sefialadas mediante una C la tienen coluvial. Las tienen una posición mixta van marcadas con AC.

Tabla 3. LOCALIZACIÓN DE LAS POBLACIONES

Nº	Nombre de la población	Intervalo de latitud	Intervalo de longitud
1	Delisi (C)	41º43´27,3´´- 41º43´38,4´´	44º42´14,3´´- 44º42´18,3´´
2	Shirikhevi (A)	41º57´50,5´´- 41º57´53,6´´	44º43´0,1´´- 44º43´17,6´´-
3	Baginchala (C)	42º2´17,3´´- 42º1´16,9´´	44º44´17,6´´- 44º44´52,5´´
4	Pantano de Zhinvali (AC)	42º8´39,8´´	44º45´58,8´´
5	Meneso (C)	42º2´17,3´´- 42º7´24,8´´	44º40´31,6´´- 44º46´31,5´´
6	Ninotsminda (C)	41º44´17´´- 41º44´20,8´´	45º17´5,9´´- 45º17´8,6´´
7	Kvetari (C)	42º3´17´´ - 42º3´38,2´´	45º6´17,6´´- 45º6´47,6´´
8	Sabue (C)	42º2´53,4´´- 42º3´21,4´´	45º7´8,2´´- 45º7´32,1´´
9	Chachkhriala (AC)	42º2´42,5´´	45º9´2,4´´
10	Samebis seri (AC)	41º56´29,1´- 41º56´32,7´´	45º46´2,1´´- 45º46´30,6´´

La altitud de las poblaciones suele estar situada entre 700 y 900 m. La mayor corresponde a la villa de Meneso, en el distrito de Dusheti, que se situa a 927m.

Referente al sexo de las parras, todos los ejemplares encontrados eran dioicos. Los masculinos con *flor masculina pura*, mientras que los femeninos tenian gineceo y 5 estambres reflejos. Como ocurre en las parras europeas, existe una gran diversidad foliar en cuanto a tamafio y contorno. Generalmente, las plantas masculinas suelen tener hojas de menor tamafio y mas divididas que las femeninas, como ocurre en Andalucia (Ocete et al., 2007). Por otra parte, se observa una gran variedad de tamafios del seno peciolar, que, en general, suele oscilar entre abierto y muy abierto.

Reespecto al fruto, dentro de una misma población aparecen racimos con bayas subesfericas, muy similares a las de las viniferas de Europa occidental y siempre de color tinto. Mas ocasionalmente, se encuentran racimos con bayas mas elongadas, subelipticas, de mayor volumen que las anteriores, que podrian estar emparentadas con las *proles póntica* y/u *orientalis* (Figuras 5 y 6).

Tutores y flora acompafiante

Acer campestris, Carpinus caucasica, Carpinus orientalis, Celtis caucasica, Celtis australis, Cichorium intybus, Clematis vitalba, Cornus mas,,Cornus australis, Corylus avellana, Cotinus coggygria, Crataegus monogyna, Berberis vulgaris, Berberis iberica, Diospyrus lotus, Fagus orientalis, Ficus carica, Fraxinus sp., Hedera helix, Hedera caucasigen, Hippophae rhamnoides,

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Juglans regia, Ligustrum vulgare, Malus orientalis, Mespilus germanica, Morus sp., Origanum vulgare, Paliurus spina-christi, Platanus, Populus tremula, Prunus avium, Prunus divaricata, Pyrus caucasica, Punica granatum, Rosa canina, Rubus sp, *Sambucus ebulus, Salix sp., Smilax aspera, Quercus sp., Ulmus sp.*

Varias de las especies citadas aparecen frecuentemente en los habitats de la vid silvestre en Europa. Como se dijo en la introducción, existen muchos parentales de frutales cultivados. Los frutos de *C. mas* son muy apreciados para la fabricación de mermeladas y salsas en la región, igualmente ocurre con los de *Pr. divaricata* y zarzamora.

Cabe resaltar que el arbusto caducifolio *P. spina-christi*, internacionalmente conocido como Crhist'thorn o Jerusalem thorn, suele ser el tutor mas comun de las parras en aquellas poblaciones situadas en posición coluvial con vegetación mas xerófita, principalmente en el caso de la de Delisi.

Fitófagos y patógenos

En ningun caso se han detectado sintomas de filoxera radicicola, como ocurre en las poblaciones silvestres europeas. Pese a que las parras son sensibles a este homóptero, al igual que las variedades euroasiaticas de cultivo, el suelo mojado durante buena parte del afio en todas las poblaciones impide el arraigo de la plaga. El insecto fue detectado en Rusia meridional en 1863 (Negrul, 1952); dieciocho afios mas tarde se describieron sus sintomas en los vifiedos de la provincia georgiana de Abkhazeti, cerca de la ciudad de Sokhumi. Entre 1889 y 1891 el problema sanitario se habia extendido a casi toda la Georgia occidental. En el este del pais fue descubierta en 1893 en la provincia de Kartli. Entre 1906 y 1910 se encontraron las primeras infestaciones de raices en Kakheti (Kantaria y Ramishvili, 1983).

La presencia de agallas es bien patente en todas las colecciones ampelograficas y campos de pies madre. Asimismo, junto a vifiedos antiguos, pueden encontrarse en los bordes portainjertos con fuerte grado de infestación gallicola. Estos eran plantados para poder disponer de material para reponer o aumentar el numero de vides de las explotaciones tradicionales.

Son muy abundantes los sintomas causados por el acaro de la erinosis, *Colomerus vitis* (Pagenstecher) (Acari, Eriophyidae), en todas las poblaciones, donde la practica totalidad de los ejemplares presentan diversos niveles de ataque. Menos frecuentes son los sintomas de acariosis, *Calepitrimerus vitis* (Nalepa) (Acari, Eriophyidae), que afectan en torno a un 57% de los ejemplares examinados. Ambas especies son, tambien, muy frecuentes en las poblaciones de toda Europa, por lo que puede pensarse, que de los ejemplares silvestres pasaron al cultivo, durante el proceso de domesticación (Figura /).

Respecto al oidio, conocido como *natsari* y mildiu, denominado *tchraki*, aparecen sintomas en todas las poblaciones, preferentemente sobre las hojas y brotes tiernos. Mas raros son los ataques al racimo.

La presencia de oidio viene documentada desde la decada de 1850 en las provincias de Guria y Samegrelo (Georgia occidental), de donde se extendió alresto del territorio. El mildiu comenzó a avistarse al final del s. XIX (Kantaria y Ramishvili, 1983; Encyclopedia, 1986). Al reves que en el caso de los acaros, los patógenos norteamericanos pasaron del vifiedo a las poblaciones silvestres.

No se han detectado sintomas de pudrición en las raices de las parras.

Tampoco se observan problemas causados por hongos de madera.

La prospección de parasitos pone de manifiesto la total convergencia sanitaria entre las vides silvestres de Georgia y las de la Peninsula Iberica, segun el listado de plagas y enfermedades de Ocete *et al*. (2007).

Vitaceas exóticas invasoras

Como consecuencia de la llegada de la filoxera, se importaron varias especies norteamericanas, como *Vitis rupestris*, *Vitis riparia*, *Vitis berlandieri* etc. Tambien hibridos entre vid europea y americana, asi como hibridos productores directos.

Las observaciones realizadas ponen de manifiesto, que la variedad Isabella, un hibrido productor directo norteamericano, es la mas frecuente. Dicha planta tambien es la mas extendida en el vifiedo tradicional del archipielago de las Azores y varias regiones sudamericanas, pese a que la OCM del vino indica que sólo puede producirse este alimento a partir de bayas de *Vitis vinifera*. Por toda Georgia pueden verse parras de esa clase ornamentando jardines y casas, dada su resistencia a los parasitos norteamericanos.

En el entorno de la costa del Mar Negro son muy frecuentes las cunetas y monticulos con restos de vegetación autóctona invadidos por dicha vitacea, que puede provocar problemas medioambientales como los que se vienen produciendoen toda Europa con especies exóticas norteamericanas, como es el caso del Valledel Danubio (Terpó, 1974), la Comunidad Valenciana (Laguna 2003), Catalufia, Cerdafia o Andalucia (Ocete et al., 2007).

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Figura 1. Cerámica antigua relacionada con el consumo de vino.

Figura 2. Relieve con vides en el exterior de una iglesia.

Figura 2. Estación de Viticultura y Enología de Telavi, donde estuvo trabajando Negrul.

Figura 4. Colección de viníferas autóctonas georgianas.

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Figuras 5 y 6. Diversos tipos de racimos femeninos de vid silvestre.

Figura 7. Hoja con síntomas de erinosis y mildiu

Chapter 8

Grape Must and Wine

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Article

Comparison Between the Grape Technological Characteristics of *Vitis vinifera* subsp. *sylvestris* and subsp. *sativa*

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Abstract: Wine has been produced in Georgia since the 6th millennium BC. The processes of cultivar selection and breeding started with wild grapes *Vitis vinifera* L. ssp. *sylvestris* Gmel. and included multiple introgression events—from the wild to domestication. This article aims at improving the knowledge concerning the history of winemaking through a comparison of the *Vitis vinifera* subsp. *sylvestris* and subsp. *sativa*. Grapes of *Vitis vinifera* subsp. *sylvestris* were grown in an ampelographic collection and vintages 2017–2020 were analyzed. The obtained data were compared to a wider dataset available in literature concerning *Vitis vinifera* subsp. *sativa*, demonstrating the central role of grape morphology in the domestication process. This evidence suggests that the technological value of the cultivars played an important role in the selection process. In vintages 2017, 2018, and 2019, wines were produced with *Vitis vinifera* subsp. *sylvestris* grapes and compared with Cabernet Sauvignon and Saperavi vinifications. For all the vintages, the fermentations took shorter time for wild grape, despite the highest content of total phenols. Learning from the past, *Vitis vinifera* subsp. *sylvestris* might still be an interesting genetic resource for future breeding programs. Furthermore, the possible combination of wild and domesticated grapes can make possible the production of wines with long ageing, exalting their own characteristics.

Keywords: wild grapes; Caucasus; Neolithic wines; genetic resources; grapevine domestication; viticulture; winemaking

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1. Introduction

A recent bio-archeological multidisciplinary research confirmed that the earliest evidence of wine production from grapes (*Vitis vinifera* L.) in large-capacity jars was in the Georgian territory [1]. The research demonstrated that Neolithic *Shulaveri-Shomu Tepe* culture grew grapevine plants near their villages in 6th millennium BC, as confirmed also by numerous pollen grains of grapes extracted from the archaeological sites of *Gadachrili Gora* and *Shulaveri Gora* since 2014 [2]. According to this data, we have to consider that grapevine domestication, with the processes of cultivar selection and breeding, was initiated 8000 years ago in the South Caucasian area.

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It is not fully understood how Neolithic population managed grapevine cultivation. Based on the available information of later historical periods, we can also assume that the Neolithic people used to protect and improve the wild grapevine populations, together with their support trees, by cleaning the surrounding forest from trees and shrubs in competition with grapevines for light and soil resources (nutrient and water). The management of these wild grapevine populations can be considered as the first stage of grape domestication named as 'Embryonic viticulture' [3].

The domestication of grapevine *Vitis vinifera* L. is still to be clarified. Based on the theory proposed by Vavilov [4], the crucial role of *Vitis vinifera* wild relative is the starting point of the process. The wild relative of the Eurasian cultivated grapevine *Vitis vinifera* ssp. *sativa* DC. is the wild grape *Vitis vinifera* ssp. *sylvestris* Gmel. Actually, these two taxa are so close to each other that botanists recognize them as a sub-species of the same Linnaeanum species. Beside the wild grapevine role in initiating the domestication process, it took an important follow-up part in different historical periods, regions, and cultures, for both winemaking and possibly multiple introgression events from the wild to the domestic compartment. This role is well documented, thanks the possibility of tracing the grape domestication through the botanical remains, mainly the seeds, from archaeological sites. An updated table concerning the archaeobotanical records of grapevine in Georgia is reported in Table S1. The well-established method used to distinguish wild and cultivated grapevine seeds is based on a range of morpho-biometric criteria, including the ratio of width and length [5]. More recently, several morphometric measurements can be processed by multivariate analysis [6]. In the initial stage of domestication, in the Early Neolithic period, the wild morphotype was usually found in the archeological sites of Caucasus. The presence of a wild seed morphotype, alone or together with domesticated morphotype, has also been dated in the following protohistoric and historic periods, until the Middle Ages, not only in Caucasus, but also in Greece, Sardinia, and France [6–9].

Archaeologists mainly infer to the simultaneous presence of wild and domestic grape seed morphotypes as a marker of primary or secondary grapevine domestication centers or as a consequence of the introduction of domesticated grapevines in regions where wild grapevines were already proto-cultivated [6,7]. According to these authors, wild grapevines might have been cultivated and used to make wine. With cultivation, we refer to grapevine management, including pruning, selective propagation, and planting to improve fruit production. Cultivation of the wild grapevine might be considered as one of the first steps in the domestication process [9]. This exploitation provides a solid basis for the initial periods of grape cultivation, but the presence of both wild grape seeds with cultivated morphotype in the following historical periods can be ascribable to other reasons. According to the literature, the probable reasons why wild grapes were used for winemaking together with domestic grapes could be: (i) the abundant availability of wild grapes in the forest and wild vegetation of Europe and minor Asia until the mid-19th century [10,11]; (ii) the scarcity of grapes from cultivated vineyards due to economic and political instability in Sardinia during the 14th century [12], in Georgia in the 18th century [13], in Germany and Italy until recent times [14–16]; (iii) the improved durability, taste, and flavor of wines obtained with wild and cultivated grapes [14]; and (iv) the production of vinegar that was one of the main products used with salt for food preservation in the past [14]. Moreover, (v) wild grapevines were also collected and employed as a medicinal plant [14,17].

Currently, wild grapevines are considered as a rare and endangered plant subspecies, despite the scientific community agreeing to consider *Vitis vinifera* L. subsp. *sylvestris* Gmel. as a precious genetic resource [18].

The production of wine with wild grapevines, known also as 'wild wine', can find justification and support due to their oenological traits. Even if certain characteristics seem to be of particular interest from a winemaking perspective, data concerning wines produced with wild grapes is still limited to make definitive assumptions. Nonetheless, previously published research has showed promising results. The wine made from the wild grapes of the river Ega (Santa Cruz de Campezo, North Spain) was characterized by high color

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intensity (26.57) and total acidity (19.3 g of tartaric acid/L) that the authors indicated of importance thinking about breeding of red cultivars in areas under a temperate climate [19]. Similarly, wild wine produced in Sardinia (Italy) had good acidity and color intensity [12]. Arroyo-García et al. [10] made wine using wild grapes from Rivera de Huelva (Andalusia, Spain); the authors indicated that microvinification led to a wine with good acidity and medium color intensity, two interesting characteristics in a warm climate. These traits of wild wines can be considered the two main characteristics for improving viticulture in current climatic conditions [20]. Lara et al. [14] reported that wild wine showed ethanol content up to 14.5% (*v/v*), good degree of acidity with pH values in the range 3.1–3.5, and a high level of total polyphenols (about 80 g/L), suggesting the suitability of must from wild grape for prolonged winemaking process. More recently, a study of seven accessions of wild wines made with grapes harvested in the forests of the South Caucasus countries, including Armenia, Azerbaijan, and Georgia, also demonstrated the diversity of enological parameters, providing the idea that the must of wild grape could be used to improve traditional wines, resulting in color intensity [21]. This data also suggests that the possible combination of wild and domesticated grapes can make a wine suitable for long ageing.

However, the wines made with wild grapes collected in the forests can only provide general information about the wild wines [21,22]. Various limiting factors, such as not-uniform berry maturation, geographical differences in plant locations, and birds picking ripe berries, can limit the effective demonstration of the maximum enological potential of wild grapes. The harvest of grapes cultivated in vineyards allows to overcome these constraints. Derosas et al. [23] studied the wines prepared from five accessions of wild grape of Sardinia located in a field collection (AGRIS Sardinia, Cagliari). The authors showed the wines had an adequate amount of ethanol and the variability of certain enological characters (like polyphenols) made the wild grapes interesting for winemaking purposes as well for further enological investigation.

Due to the important innovation in molecular biology of the last decades, research is mainly focused on plants' genetic aspects. In fact, it has been shown that the identification and study of genetic diversity using SSR markers is the most studied aspect concerning wild grapevine populations [18]. To add to current knowledge, this study aimed to provide eno-carpological characteristics of wild grapevine *Vitis vinifera sylvestris* from Georgia. These data were than compared to the similar data of the large set of cultivated grapevines of *Vitis vinifera sativa* from Georgia and other European countries. Moreover, the composition of wines produced with wild and cultivated grapes was evaluated in order to better understand the role of grape breeding in enology during millennia. The interest of studying wines made from wild grapes in comparison to cultivated grapes becomes evident by analyzing both grapes and wines. The long-term goal will be to increase the knowledge about wild grapes and wines and to evaluate the suitability and potentiality of wild grapes for wine production.

2. Materials and Methods

2.1. Experimental Design: Experimental Site Description, Plant Material, and Maintenance

The experiment was conducted in the *Vitis vinifera* subsp. *sylvestris* collection established during 2014–2016, facilitated by the research program of the National Wine Agency for the "Study of Vine and Wine Culture of Georgia". The plant materials grown in that vineyard were discovered during expeditions to the territory of Georgia in 2003 under the framework of various national and international projects. Molecular fingerprinting based on SSR and SNPs markers have been used to identify the true-to-type of *Vitis sylvestris* accessions [24].

This vineyard belongs to the Jighaura collection (FAO code GEO038) named after academician S. Cholokashvili of LEPL Scientific-Research of Agriculture (Mtskheta, Kartli Province of Eastern Georgia). The *Vitis vinifera* subsp. *sylvestris* collection site (Latitude 41.90, Longitude 44.76, Elevation 513 m a.s.l.) accumulates 2100–2350 °C of Growing Degree Days (GDD) and 540–590 mm of average annual precipitation [25]. The soil of the

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site is meadow brown, and it has good physical properties and the ability to retain water. The content of lime increases deeper into 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) x 1.3 m (between plants). The pruning system is Double-Guyot (20–24 buds/vine). The soil is managed with a natural grass-cover system. If necessary, a drip-irrigation system is available. Nutritional supply and pesticide control are managed to guarantee the good development and production of plants, as well as their healthy conditions during all vegetative seasons. All the vines are grafted on Kober 5 BB (*Vitis berlandieri* x *Vitis riparia*) rootstocks.

The collection maintains 60 wild grapevine accessions (3–5 plants/accession), including both male and female plants. However, only plants having female type of flowers and producing grapes were considered in this research.

2.2. Eno-Carpological Description

The analyses were carried on the 2017–2020 period. Most of the accessions were studied for 3–4 vintages, however, this number varied depending on the availability of grapes in each accession. Details concerning the number of measurements for each parameter are available in Table S2.

The standard phenotyping protocol proposed by the COST action FA1003 "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding" has been adopted for eno-carpological evaluation of wild accessions [26–29]. Briefly, 3 replications of 3 representative bunches for each accession were collected at maturity stage (upon stable sugars concentration) and weighted. From each replicate, 10 berries were selected and their diameters measured. These berries were also used to quantify the berry weight, skin weight, seed number, and weight. Skins and seeds were extracted in a hydrochloric ethanol solution (ethanol/water/hydrochloric acid 37% 70/30/1), to quantify the total phenolic and anthocyanin concentrations. Phenols were analyzed separately for skin and seed extracts, using the Folin–Ciocalteu reagent. The absorbance for total anthocyanins (at 540 nm) and total polyphenols (at 700 nm) was measured by using a UV-1100 Spectrophotometer (Jiangsu, China) and respectively expressed as malvidin-3-O-glucoside (mg/kg of grape) and (+) catechin (mg/kg of grape) concentration [30,31]. The surplus of the bunches was pressed to obtain musts. The total soluble solids (°Brix) were measured by a digital refractometer and total acidity by titration with sodium hydroxide 0.1 N with bromothymol blue as the indicator.

2.3. Wine Production and Characterization

During vintages 2017, 2018, and 2019, wines were produced with *Vitis vinifera* subsp. *sylvestris* grapes. Due to the low yield of wild grapevines, all the productive accessions were mixed. As comparison, aliquots of Cabernet Sauvignon and Saperavi grapes were also harvested at the Jighaura collection of the LEPL Scientific-Research Center of Agriculture and microvinifications were carried out. The amounts of the three grape cultivars considered and vinified for each vintage are reported in Table S3.

Vinifications were carried out by using the red winemaking method (with grape skin maceration) [32]. Briefly, grapes were hand-harvested and destemmed to remove the stalks. Crushing was carried out manually and the obtained musts were added with potassium metabisulfite (60 mg/L) in order to prevent spontaneous and undesired fermentations. Inoculum was performed with a commercial yeast (IOC 18-2007, 0.2 g/L) that was prepared by dissolving the yeast powder in 100-times volume of water. The yeast suspension was kept at 36 °C for 15 min under shaking, and the same amount of grape must was added in order to adapt the yeast cells to the temperature of must. After 10 min, the yeast suspension was added to musts contained in three separate small tanks (10–20 L). The alcoholic fermentation was carried out at 20 ± 2 °C and it lasted up to 25 days (Table S2). Fermentations were conducted to dryness. At the end of the alcoholic fermentation, grape pomaces were separated by pressing and the obtained wines were added with potassium

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metabisulfite (60 mg/L). The wines were kept in the microvinification tanks at 15–20 °C for 6 months; after stabilization and clarification, they were racked and bottled in green bottles (750 mL) closed with an agglomerated cork cap after the addition of potassium metabisulfite (60 mg/L). The wines were stored at 15–20 °C and analyzed six months after the bottling.

The analyses of musts and wines were carried out following the official protocols reported by the International Organization of Vine and Wine (OIV). In particular, the analyzed parameters in both musts and wines were sugar content (g/L; OIV-AS311-01A), pH (OIV- MA-AS313-01) and total acidity (g/L of tartaric acid; OIV- MA-AS313-01). Volatile acidity (g/L of acetic acid; OIV- MA-AS313-02), ethanol (% (*v/v*); OIV- MA-AS312-01A), malvidin diglucoside (mL/L; OIV- MA-AS312), total phenol content (mg/L of catechin; OIV-MA-E-AS2-10-INDFOL; spectrophotometer SP-Carry-50—Los Angeles, CA, United States), and total dry extract (g/L; OIV-MA-AS2-0315-11; equipment- KNAUER thermo chromatography) were determined in wine samples.

2.4. Statistic Data Processing

The software SPSS (SPSS, Chicago, IL, USA) version 22.0 was used for the statistical data processing.

The description of the Georgian population of *Vitis vinifera* subsp. *sylvestris* was compared to the *Vitis vinifera* subsp. *sativa* dataset collected during the COST action FA1003 "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding" [26–28,33]. Differences and similarities are shown overlapping the frequency distribution graph of the two populations for each studied parameter. Descriptive analysis (average, minimum, maximum, quartiles—25, 50, 75) of the Georgian *Vitis vinifera* subsp. *sylvestris* population was compared to the data available in literature concerning the Georgian [33] and Euro-Asiatic [26] *Vitis vinifera* subsp. *sativa* populations.

Data related to the composition of must and wine samples were averaged among vintages as only negligible differences were found. One-way ANOVA was carried out and significant differences among must and wine samples produced from different grapes were determined by F-test (LSD) considering $p < 0.1$ and $p < 0.05$.

3. Results

3.1. Grape Characterization

Vitis vinifera subsp. *sylvestris* had smaller fruits than *Vitis vinifera* subsp. *sativa*. The bunches were smaller (Figure 1a), made by smaller berries (Figure 1b–d). However, the shape of the berry remained similar, with a dominance of round berries (Figure 1e). Obviously, the smaller berries had a higher ratio of surface with respect to the sphere volume and, thus, the contribution of skins to the total berry weight was higher in the subsp. *sylvestris* (Figure 1f).

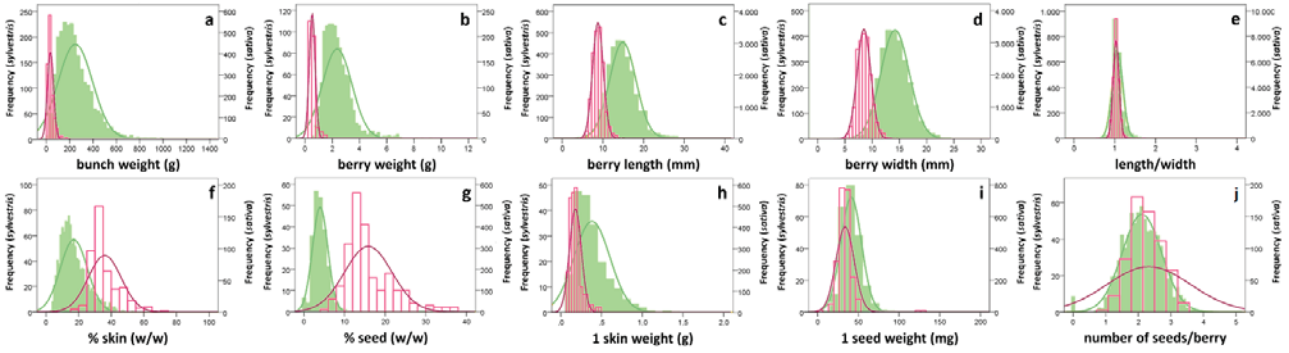

Figure 1. Carpological characteristics of *Vitis vinifera* subsp. *sylvestris* (in pink) and subsp. *sativa* (in green). *Vitis vinifera* subsp. *sativa* data are already published in Rustioni et al. [26]. (**a**): bunch weight (g), (**b**): berry weight (g), (**c**): berry length (mm), (**d**): berry width (mm), (**e**): length/width, (**f**): % skin (*w/w*), (**g**): % seed (*w/w*), (**h**): one skin weight (g), (**i**): one seed weight (mg), (**j**): number of seeds/berry.

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The number of seeds per berry was similar among the subspecies (Figure 1j), with a slightly larger number in the subsp. *sylvestris* (Table S2). Nevertheless, despite the seed weight being generally lower in subsp. *sylvestris* (Figure 1i), the contribution of seed to the total berry weight was higher in the subsp. *sylvestris* (Figure 1g) due to the smaller berries with less pulp.

The *Vitis vinifera* subsp. *sylvestris* musts were more concentrated than *Vitis vinifera* subsp. *sativa* in both sugars and acids (Figure 2a,b).

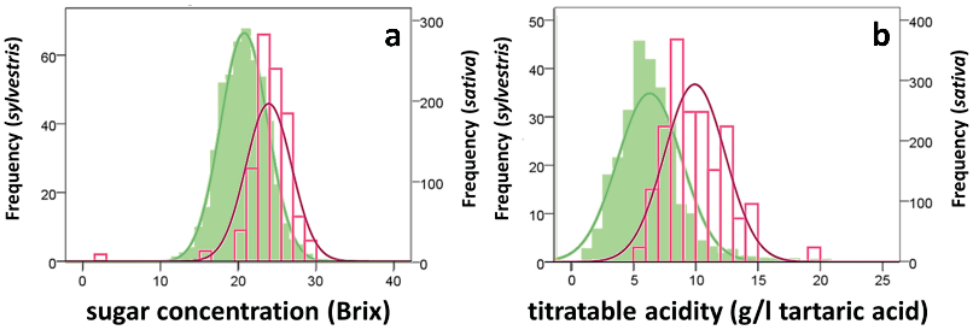

Figure 2. Technological parameters of the must of *Vitis vinifera* subsp. *sylvestris* (in pink) and subsp. *sativa* (in green). *Vitis vinifera* subsp. *sativa* data are already published in Rustioni et al. [26]. (**a**): sugar concentration (Brix), (**b**): titratable acidity (g/L tartaric acid).

The grapes of *Vitis vinifera* subsp. *sylvestris* had a higher concentration in anthocyanins than *Vitis vinifera* subsp. *sativa* (Figure 3a). However, this is mainly due to the carpological features of the grapes, with higher proportions of pigmented skins in subsp. *sylvestris*. In fact, the accumulation of pigments in the skin tissue was very similar among the two subspecies (Figure 3c) and, thus, a small *sylvestris* berry had a lower amount of anthocyanins (Figure 3b), having a smaller skin.

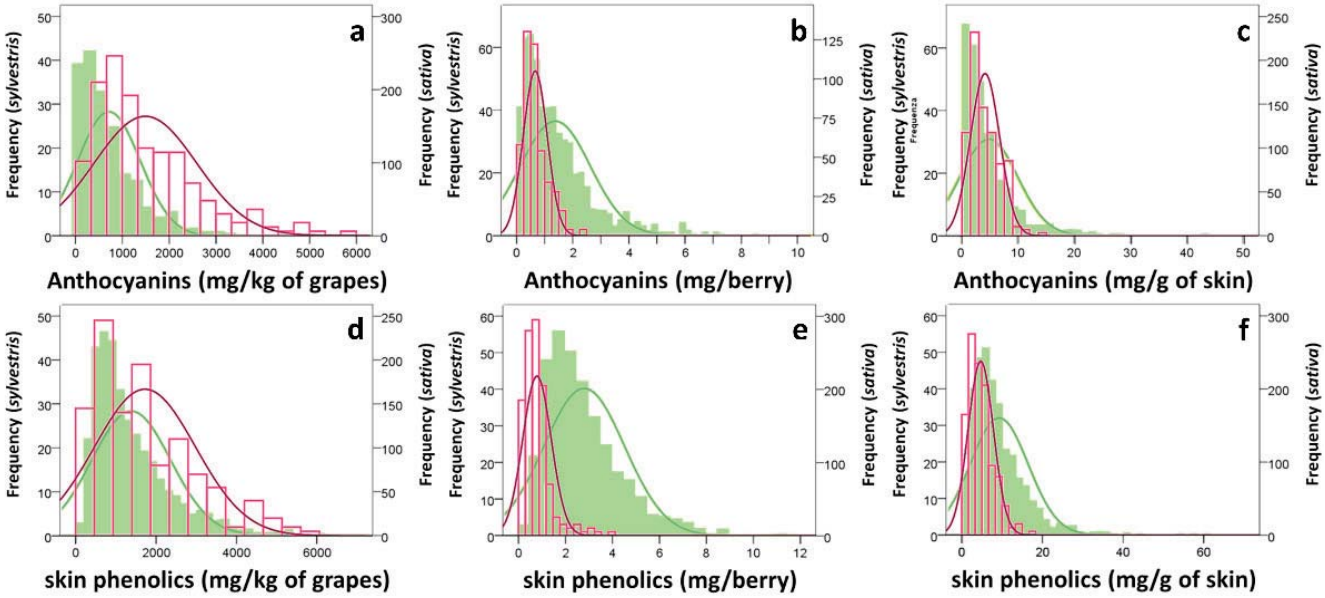

Figure 3. Anthocyanins and phenolics in skins of *Vitis vinifera* subsp. *sylvestris* (in pink) and subsp. *sativa* (in green). *Vitis vinifera* subsp. *sativa* data are already published in Rustioni et al. [26]. (**a**): anthocyanins (mg/kg of grapes), (**b**): anthocyanins (mg/berry), (**c**): anthocyanins (mg/g of skin), (**d**): skin phenolics (mg/kg of grapes), (**e**): skin phenolics (mg/berry), (**f**): skin phenolics (mg/g of skin).

Concerning the skin phenolics, the lower concentration in the small *sylvestris* berries (Figure 3e) was exacerbated by a lower ability of the skin tissue in their synthesis (Figure 3f).

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Nevertheless, the higher skin percentage of the berry weight (Figure 1f) ensured a slightly higher phenolic concentration in *sylvestris* grapes (Figure 3d).

The grapes of *Vitis vinifera* subsp. *sylvestris* had a higher concentration in seed phenolics than *Vitis vinifera* subsp. *sativa* (Figure 4a), due to the higher seed percentage of the berry weight (Figure 1g), despite the generally lower ability of *sylvestris* seeds to accumulate phenolics (Figure 4b–d).

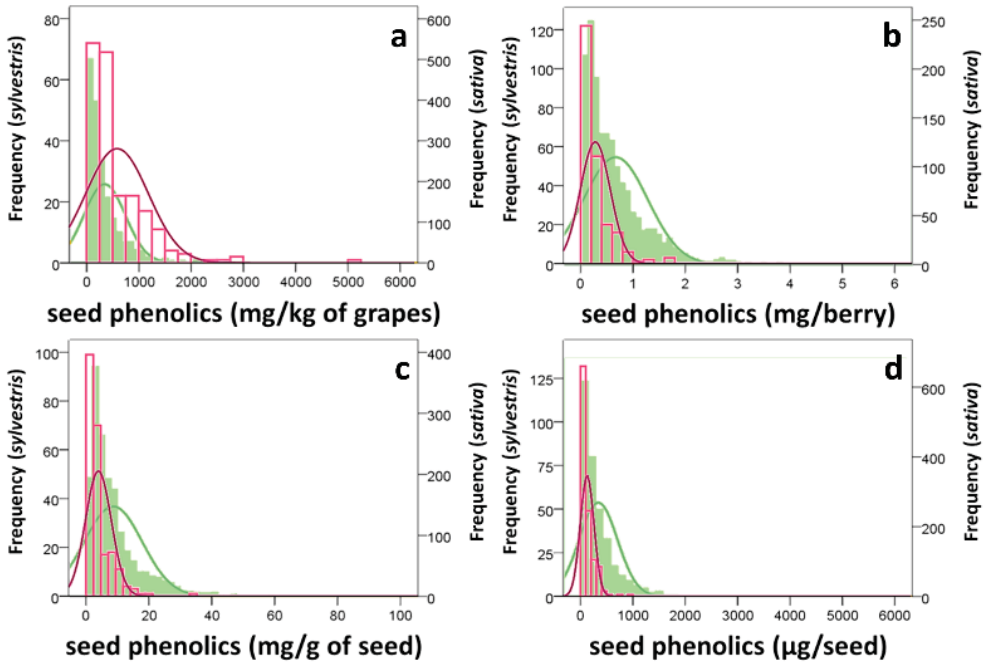

Figure 4. Seed phenolics in *Vitis vinifera* subsp. *sylvestris* (in pink) and subsp. *sativa* (in green). *Vitis vinifera* subsp. *sativa* data are already published in Rustioni et al. [26]. (**a**): seed phenolics (mg/kg of grapes), (**b**): seed phenolics (mg/berry), (**c**): seed phenolics (mg/g of seed), (**d**): seed phenolics (µg/seed).

Despite the lower accumulation of phenolics in the small *sylvestris* berries (Figure 5d), the carpological features ensured a slightly higher total phenolic amount in *sylvestris* grapes (Figure 5c). These phenolics came mainly from skins (Figure 5a), still the proportion rising from seeds was higher in *sylvestris* with respect to sativa (Figure 5a,b).

Enlarging the comparison of the *sativa* grapes cultivated in Georgia (Table S2), it is worth noticing that these grapes were characterized by carpological descriptors with values often in between the ones recorded for *sylvestris* and *sativa*, with values obviously closed to the ones recorded in *sativa*. However, Georgian cultivars were characterized by thicker skins and heavier seeds, and this has impacts on the phenolic components.

3.2. Composition of Musts and Wines

The sugar concentration of all the three musts samples investigated in this study indicated the ripeness of the grapes and the suitability of harvest time for wine production. The sugar concentration was slightly higher in wild grapes, while acidity and pH were higher in Saperavi grape (Table 1). However, no significant difference was found in the chemical parameters of must samples.

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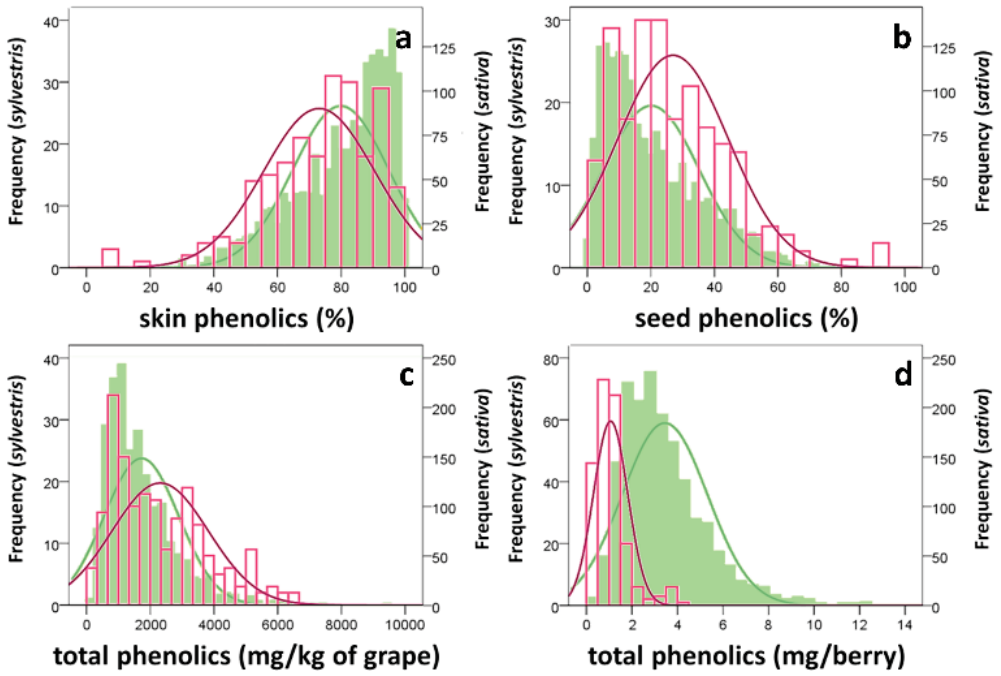

Figure 5. Total phenolics and their origin proportions in *Vitis vinifera* subsp. *sylvestris* (in pink) and subsp. *sativa* (in green). *Vitis vinifera* subsp. *sativa* data are already published in Rustioni et al. [26]. (**a**): skin phenolics (%), (**b**): seed phenolics (%), (**c**): total phenolics (mg/kg of grapes), (**d**): total phenolics (mg/berry).

Table 1. Sugar concentration, total acidity, and pH for the musts produced with wild grape, Cabernet Sauvignon, and Saperavi grapes. Data is expressed as average ± standard error of the three vintages investigated. Different letters mean significant differences (F-test). #: LS, level of significance: ns, non-significant.

Must	Wild Grape	Cabernet Sauvignon	Saperavi	LS #
Sugar concentration (°Brix)	25.1 ± 0.9 ^{a}	24.1 ± 1.4 ^{a}	22.1 ± 1.2 ^{a}	ns
Total acidity (g/L of tartaric acid)	7.8 ± 0.8 ^{a}	6.7 ± 0.7 ^{a}	9.2 ± 1.3 ^{a}	ns
pH	3.4 ± 0.2 ^{a}	3.4 ± 0.2 ^{a}	3.2 ± 0.2 ^{a}	ns

In all the microvinifications carried out on vintages 2017, 2018, and 2019, the alcoholic fermentations were completed after maximum 25 days. For all the vintages, the fermentations took shorter time in case of wild grape, while they were the longest with Saperavi grape. Only negligible differences were found in the residual sugars, except for Cabernet Sauvignon wine produced in 2018 (8.1 g/L vs. 1.8 g/L in both 2017 and 2019 wine samples). As expected, malvidin diglucoside was not detected in Cabernet Sauvignon and Saperavi wines, while its concentration was 2.5 ± 2.1 mL/L in wild grape wines. The concentration of ethanol, volatile acidity, and pH did not show any significant differences between the wine samples produced with the grapes investigated (Table 2). Total acidity was the lowest in Cabernet Sauvignon wine being significant ($\alpha = 0.05$) in comparison to both wild grape ($p = 0.013$) and Saperavi ($p = 0.022$) wines. Saperavi wines showed the lowest total dry extract, which was significantly lower. Wild grape wines had the highest content of total phenol (3.1 ± 1.1 g/L), significant for $\alpha = 0.01$ in comparison with both Cabernet Sauvignon and Saperavi wines. Nonetheless, preliminary sensory data indicated the difference was not significant for the perception of acidity or for phenolic-related attributes (i.e., astringency, bitterness, phenols) and sapidity (data not shown).

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Table 2. Chemical parameters for the wines produced with wild grape, Cabernet Sauvignon, and Saperavi grapes. Data is expressed as average ± standard error of the three (2017, 2018, 2019) vintages investigated. Different letters mean significant differences (F-test). #: LS, level of significance: ns, non-significant; *, $p < 0.1$; **, $p < 0.05$.

Wine	Wild Grape	Cabernet Sauvignon	Saperavi	LS #
Residual sugars (g/L)	3.0 ± 0.9 [a]	5.0 ± 4.5 [a]	1.9 ± 0.2 [a]	ns
Total acidity (g/L of tartaric acid)	7.1 ± 0.5 [a]	6.2 ± 0.2 [b]	7.2 ± 0.5 [a]	**
Volatile acidity (g/L of acetic acid)	0.5 ± 0.2 [a]	0.6 ± 0.1 [a]	0.6 ± 0.0 [a]	ns
pH	3.6 ± 0.0 [a]	3.3 ± 0.4 [a]	3.3 ± 0.2 [a]	ns
Ethanol (%, *v/v*)	14.2 ± 0.8 [a]	13.8 ± 1.0 [a]	13.7 ± 0.9 [a]	ns
Total phenol content (g/L of catechin)	3.1 ± 1.1 [a]	1.7 ± 0.4 [b]	1.7 ± 0.4 [b]	*
Total dry extract (g/L)	33.6 ± 3.7 [a]	31.4 ± 5.3 [a]	25.2 ± 1.9 [b]	*

4. Discussion

Coherently with most of the cultivated crops, grapevine selection during millennia of viticulture was mainly aimed at the increase of production yield, both in the field and in the winery. It means bigger bunches and bigger juicy berries with a reduced proportion of solid parts.

Our results indicate that wild grapes have a higher proportion of seeds with respect to *Vitis vinifera* subsp. *sativa* population. It is worth noticing that this result was obtained in an ampelographic collection, where the dioecious character of *Vitis vinifera* subsp. *sylvestris* was counterbalanced by the high presence of male wild and also other grapevine plants producing sufficient quantity of pollen for guaranteed pollination of female wild grapes. In fact, when the *Vitis vinifera* subsp. *sativa* population was compared to *Vitis vinifera* subsp. *sylvestris* populations grown in the wild, a lower number of seeds per berry was observed in the *sylvestris* grapes [34]. Thus, when pollination occurs, *Vitis vinifera* subsp. *sylvestris* seems to have a more performing reproductive physiology, resulting in a higher number of seeds/berry, with respect to cultivated grapes. We can suppose that, during domestication, humans selected juicy berries, with a lower percentage of seeds. This hypothesis is coherent with the domestication syndrome characteristics, that includes changes in the reproductive systems towards increased selfing (hermaphrodite flowers of subsp. *sativa*) and replacement of sexual reproduction by vegetative reproduction, maintaining the trueness to type and improving the appetizing characters [35]. In fact, the extreme case of seedless grapes was appreciated since the birth of our culture, by Greek philosophers and ancient Egyptians, and seedlessness (both parthenocarpy and stenospermocarpy) is still attracting the interest of both the industrial and the scientific communities [36]. The reproductive anatomy of cultivated grapes includes a series of steps, and partial dysfunctions could occur at different stages of the reproductive cycle, modulating the intensity of the disorders [37]. Thus, a less pressing selection on this trait may have resulted in a slightly lower number of seeds in cultivated grapes, even when seedlessness is not fully achieved.

Considering the other carpological traits, it is clear that the domestication process was focused on grape and wine production, not only selecting bigger bunches and berries, but also choosing the juiciest fruits, with a higher percentage of pulp with respect to seeds and skins. Different studies confirmed the central role of agricultural yield in the domestication process of different crop species [35,38]. Nevertheless, highlighting the different carpological proportions of pulp, skin, and seeds in *Vitis vinifera* subsp. *sativa* and subsp. *sylvestris*, we can hypothesize that the technological value of the cultivars

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also played an important role in the selection process. In addition, the technological use of cultivated plants played a central role in the selection of other crops. For example, different methods used for rice harvest imposed different selective pressures, and the same species have been domesticated for different food organs in different regions (e.g., lettuce is used for edible leaves in the Mediterranean regions and for enlarged edible stem in China) [39]. However, considering grapevine, we can suggest that this evidence confirms the predominant use of *Vitis* fruits for winemaking purposes since the birth of its cultivation and that the plant domestication evolved together with oenological technology. This hypothesis is also coherent with the archeological artifacts found in Georgia related to the ancient history of winemaking [40–42]. Finally, the central role of Caucasian territories in the grapevine domestication devoted to wine production is confirmed by the intermediate values observed in Georgian cultivated grapes between *Vitis vinifera* subsp. *sativa* and subsp. *sylvestris* [33].

We observed that in *Vitis vinifera* subsp. *sylvestris*, berry pulp generally has higher concentrations of sugars and acids, with respect to the *Vitis vinifera* subsp. *sativa*. Considering sugars, it is worth noting that this work does not deepen the dynamics of accumulation and further studies could point out subtler differences based, for example, on the mechanisms of sugar accumulation in the two subspecies or on the impact of the harvesting time on the obtained wine flavors [43,44].

Considering phenolics, the main differences between *Vitis vinifera* subsp. *sylvestris* and subsp. *sativa* should be ascribed to the differences in the carpological traits, despite specific disfunctions in the phenylpropanoid biosynthetic pathway taking away the attention of winegrowers during the selection of specific white and pink cultivars [45–49]. Of course, beside genetic characteristics, (micro-)environmental conditions and vineyard management affect the vine phenotype and grape enological potential, especially when we deal with secondary metabolisms, such as phenolics [50–53]. Unfortunately, knowledge concerning the impact of prehistoric viticulture practices on grape quality is not available. However, we can suppose that 'embryonic viticulture' management co-evolved together with plant domestication and cultivar selection.

The yield-based selection that occurred during domestication resulted in a loss of traits that could be of interest in the light of current knowledge concerning the importance of plant resilience to climate changes or modern enological objectives. These traits could be of particular interest in the perspective of new breeding purposes [54].

The domestication of grapes did not seem to play a role in the composition of musts investigated. Nevertheless, even if the sugar concentrations in musts were comparable and similar fermentation conditions were applied (i.e., starter yeast, temperature), the longer time observed for Saperavi must suggests the high acidity could slow down fermentation. Considering the chemical parameters determined in wines, that obtained with wild grape were characterized by a high total acidity and total phenol content. Other authors found that wines produced with wild grape had high acidity, making the grape suitable for growing in temperate and warm climate [10,12,19]. Moreover, the high level of phenols allowed to perform prolonged winemaking process [14].

5. Conclusions

This article enhances current knowledge of wild grape and wine. The changes that occurred in grape berries as a consequence of the domestication process involved the number of seeds, the carpological traits, as well content of phenolics. These characteristics are of particular interest for an effective response against climatic changes and the development of modern viticulture through new breeding activities.

Wines obtained with wild grape can be suitable for prolonged winemaking in which wood aging can be also expected in order to allow its evolution. The possible combination of wild and domesticated grapes can make possible the production of wine with long ageing, exalting their own characteristics. The possibility to produce wine with grape being more tolerant to the environmental stresses could represent an advantage due to the lower

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input requirements in viticulture and in terms of grape characteristics and wine quality. As a consequence, the production of high-level wine could be sustainably maintained, effectively responding to consumers' requests. The interested differences among the grapes investigated, especially for phenols, evidence the possibility of the oenological use of wild grape enriching the phenolic content and making long aging suitable even for those varieties that are poorer in mouthfeel and body.

Further investigation will study the determination of aroma profile from both analytical and sensory points of view, as well as a more detailed characterization of phenolic compounds.

Supplementary Materials: The following are available online at https://www.mdpi.com/2073-4395/11/3/472/s1, Table S1: Archaeobotanical records of grapevine in Georgia, Table S2: Comparison in the data distribution of *Vitis vinifera* subsp. *sylvestris* and subsp. *sativa*, with details of sativa plants cultivated in Georgia. *Vitis vinifera* subsp. *sativa* data are already published in Rustioni et al [26] and Georgian cultivars are described in Sargolzaei et al. [33], Table S3: Amounts (kg) of grapes vinified in vintages 2017, 2018, and 2019. The duration of alcoholic fermentation (days) is reported in brackets.

Author Contributions: Conceptualization, O.F. and D.M.; methodology, L.R. and S.K.; investigation and analyses, S.K., T.M. and O.G.; plant material, D.M. and O.G.; data elaboration and discussion, L.R. and D.F.; writing—original draft preparation, D.M., L.R. and D.F.; writing—review and editing, O.F., D.M., L.R. and D.F.; funding acquisition, O.F. and D.M. All authors have read and agreed to the published version of the manuscript.

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

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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

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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

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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).

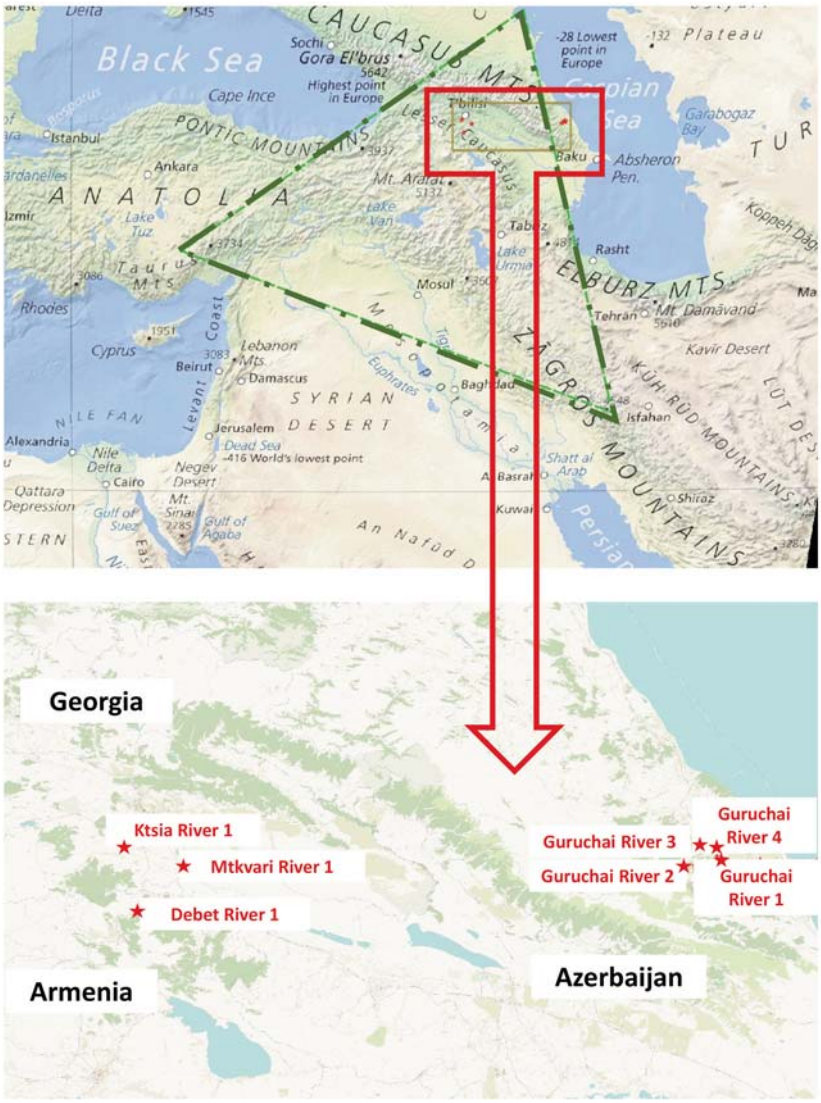

FIGURE 1. The "Vavilov's Triangle" and sampled localities.

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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.

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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

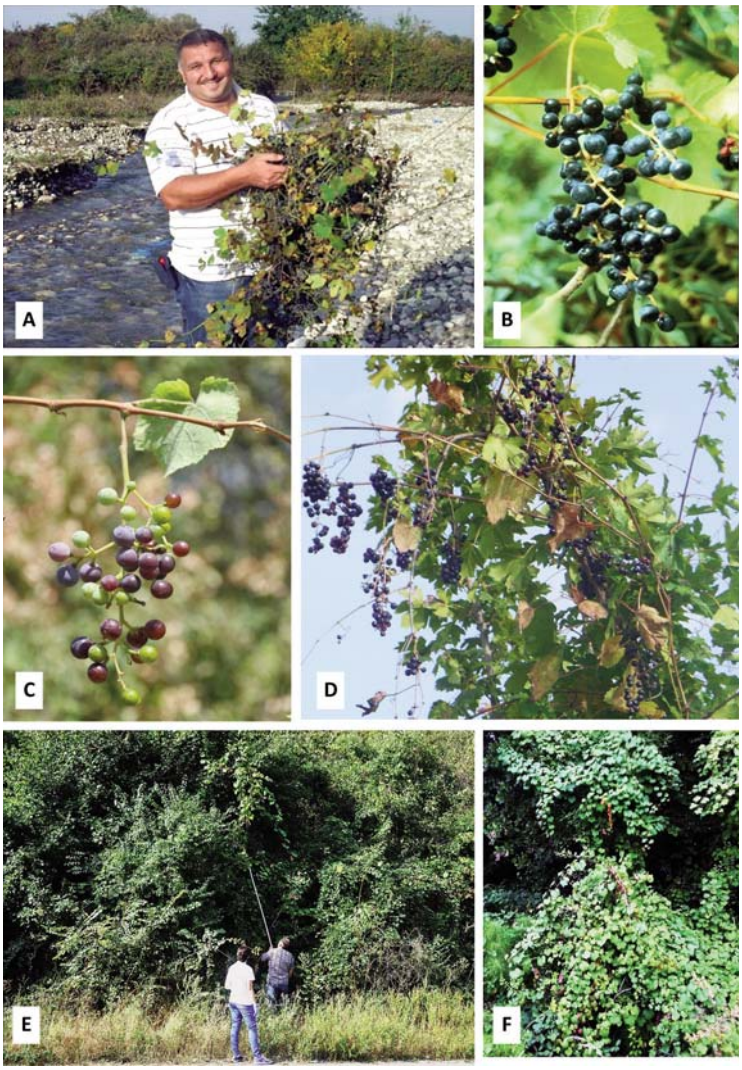

FIGURE 3. Harvest of wild grapes and habitats.
A, Harvest of wild grapes in Guruchai River (Azerbaijan); B, Ripe wild grapes, Guruchai River; C, Ripe wild grape from Ktsia River (Georgia); D, Fruiting wild grapevine in Guruchai River (Azerbaijan). E, Harvesting climbing grapevine (Georgia). F, Climbing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

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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 fermen-

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tation 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

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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.

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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

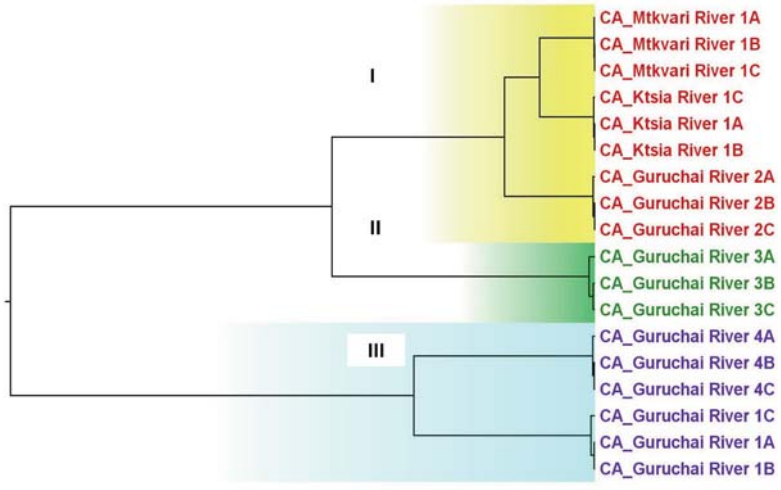

FIGURE 4. Relationships among Caucasus wine samples.
Note: Ward's minimum variance tree. A, B, C variants within each sample that were defined using mean-sd (A), mean (B), and mean+sd (C) values for each parameter).

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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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Chapter 9:

Microbiology

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Diversidad genética de levaduras aisladas a partir de uvas de Vitis vinifera ssp. Sylvestris (Gmelin) Hegi en el área Euroasiática / Genetic diversity of yeasts isolated from Eurasian populations of *Vitis vinifera* ssp. *Sylvestris* (Gmelin) Hegi

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Abstract. *Vitis vinifera* L. ssp. *Sylvestris* (Gmelin) Hegi is recognized as the dioecious parental generation of today's cultivars. Climatic change and the arrival in Europe of pathogens and pests have led it to be included on the IUCN Red List of Threatened Species in 1997. At best of our knowledge, no studies on microbial populations of grape-berry surfaces have been done. The present work has been focused on the study of yeast occurrence and diversity on grape-berries collected from wild vines. Final outputs have allowed: *i*)to obtain precise information about yeast communities; *ii)* to provide an objective framework for the classification of the broadest range of species according to their extinction risk; *iii)* to select attractive yeast strains for their biotechnological potential, offering new opportunities to winemakers. Sampling plan was performed in Azerbaijan, Georgia, Italy, Romania and Spain. In all, 3180 yeast colonies were isolated and identified as belonging to 50 species, including *Saccharomyces cerevisiae*, by 26S rDNA D1/D2 domains and ITS region sequencing. Isolates of *S. cerevisiae* were also analysed by SSR-PCR obtaining 163 different genotypes. This study highlights the biodiversity potential of pristine environments that still represent a fascinating source to face common problems in winemaking.

1. Introducción

Las poblaciones de vid silvestre euroasiática se extienden desde la región caucásica pasando por la cuenca del Mediterráneo (Turquía, Grecia, Italia, Sur de Francia y Península Ibérica) hasta el macizo del Hindu Kush (Afganistán y Pakistán) y el Magreb (Marruecos, Túnez y Argelia). Sus ejemplares pertenecen al taxón *Vitis vinifera* L. subespecie *sylvestris* Gmelin (Hegi), única especie ancestral en Europa y son parentales dioicos de las variedades de cultivo. Estas últimas son fundamentalmente hermafroditas, aunque pueden encontrarse también ejemplares femeninos, como ocurre en la región Caucásica [1]. La Península Ibérica e Itálica constituyen hoy en día uno de los principales refugios para esta subespecie durante la última glaciación. En España existen pruebas palinológicas del Pleistoceno medio en las turberas de El Padul (Granada), y en la Laguna de Las Madres (Huelva) [2, 3].

Según las referencias de Rivera y Walker [4], dentro de la Península Ibérica, las bayas de vid silvestre han contribuido directamente a la alimentación humana desde el Paleolítico. Las plantas domesticadas por el hombre son aquéllas que le sirven para su dieta o son aplicables a sus actividades cotidianas, entre ellas se encontraba esta liana. A partir de los escasos ejemplares hermafroditas aparecidos en la naturaleza como resultado de mutación, se fueron seleccionando variedades de cultivo [5]. Un estudio publicado en 2006 que lleva a cabo los diversos clorotipos y su distribución en 1201 muestras de muy diverso material silvestre y cultivado, procedente de diferentes áreas de la Península Ibérica, Itálica, Oriente Medio y Norte de África refuerza la teoría del origen policéntrico de la domesticación de la vid [6]. El artículo señala, además, que el 70% de los viñedos de la Península Ibérica e Itálica exhiben clorotipos derivados de las poblaciones silvestres de Europa Occidental, por lo que sostiene la idea sobre la existencia de una región de refugio para la vid, entre otras especies botánicas, en la Península Ibérica y cuyos genes son completamente distintos de las variedades cultivadas. Parece ser que el proceso de domesticación fue acelerado y guiado en primer lugar por la influencia cultural, y después, por las aportaciones directas de las actividades que realizaron los colonos fenicios, griegos y púnicos en la cuenca del Mediterráneo occidental. Se puede pensar, por lo tanto, que en las zonas de distribución de la vid silvestre, la introducción en primer lugar del consumo del vino y, posteriormente, de la viticultura, se sobrepusieron al preexistente sustrato de cultura local, caracterizado por una fase de proto-domesticación de la vid [7].

Antes del empleo de las variedades hermafroditas cultivadas, los racimos silvestres constituyeron la materia prima del vino. Probablemente el hombre, durante milenios, llevó a cabo un proceso de frutalización con el fin de aumentar la presencia de este recurso natural en

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sus alrededores en detrimento del uso de la vid silvestre. Con el desarrollo y aumento de las comunicaciones, la acción humana ha ido progresivamente destruyendo los hábitats de esta planta trepadora de forma directa o indirecta, mediante obras públicas (embalses, puentes, trazado de carreteras), expansión de las zonas agrícolas y explotaciones forestales, así como con la limpieza de las cunetas de las carreteras [7], por lo cual a día de hoy sólo quedan algunos reductos de poblaciones en la Península Ibérica, Itálica, Helénica, Anatolia y región Transcaucásica. Arnold et al. [8] concluyen, además, que la vid silvestre en Europa está más afectada que en otros lugares del Mundo debido a que quedan muy pocos refugios para la misma y la tasa de supervivencia es muy baja, pues los intentos de recuperarla mediante trasplantes y reforestación suponen un elevado coste económico y con sin éxito. Así, todo ese creciente impacto ambiental negativo ha llevado a la vid silvestre a figurar como especie amenazada en la lista roja publicada por la Unión Internacional para la Conservación de la Naturaleza (IUCN, 1997) [9].

En la Península Ibérica, los hábitats que albergan todavía un mayor número de parras silvestres son los bosques de ribera (Sierra Norte de Sevilla, Huelva y Parque Nacional de Doñana), ya que las parras son hidrófilas y toman árboles y arbustos como tutor, dado su carácter heliófilo. Los principales tutores corresponden a diversas especies de los géneros: *Acer*, *Alnus*, *Crataegus*, *Ficus*, *Fraxinus*, *Olea*, *Populus*, *Quercus*, *Retama*, *Rubus*, *Ulmus*, *Rubus*, entre otros. Asimismo, aparecen en diversas zonas coluviales de clima húmedo, como es el caso de la costa cantábrica. Hay refugios de vid silvestre en el Norte y en el Sur de la Península Ibérica. La supervivencia de esta subespecie se ve también expuesta a las actividades agrícolas, aves (especialmente alcedinos) y otros factores humanos, además de la filoxera [7]. Según Böhm [10], la vid silvestre que alberga la Península Ibérica también presenta particularidades genéticas diferentes a otras de su misma especie en otras regiones de la cuenca Mediterránea. Este autor denomina a las vides presentes en la península Ibérica (España y Portugal) como vides del "polo ibérico" y a las presentes en la península Itálica como vides del "polo ítalo-etrusco". En Italia se distribuyen por diferentes regiones siendo las principales Piemonte, Lombardía, Cerdeña, Calabria, Basilicata y Sicilia [11].

Aparte de los usos tradicionales citados, la vid silvestre constituye un importante recurso fitogenético que alberga una importantísima diversidad fitogenética, con la que hay que contar para futuros programas de mejora de viníferas y portainjertos, así como para la reforestación de ecosistemas naturales. Actualmente, mediante marcadores moleculares, se abordan estudios sobre la contribución genética de las vides silvestres a las variedades de cultivo características de algunos puntos de la región Mediterránea. Sin embargo, bajo nuestro conocimiento, aún no existen estudios que determinen la ecología de microorganismos asociados a la uva de vides silvestres, siendo de especial relevancia aquellos microorganismos implicados en la fermentación vínica y maloláctica, levaduras, bacterias lácticas o bacterias productoras de vinagre (bacterias acéticas). Por ello, pensamos que preservar la vid salvaje y su consecuente biodiversidad de especies microbianas es de enorme importancia no solo por su interés actual para la industria enológica y vinagrera tan importante en los países del Mediterráneo así como en otros del Mundo, sino también para asegurar la conservación de un *pool* de genes de gran importancia tecnológica para la industria alimentaria en general y mantener estos recursos genéticos microbianos ante la inminente amenaza de extinción de la vid silvestre y crear nuevos estilos de vinos y vinagres que coloquen a España e Italia de nuevo a la cabeza en innovación enológica. En este sentido se hacen necesarios estudios que ayuden a cubrir ese "vacío de conocimiento". La extinción de las pocas poblaciones de vid silvestre en Europa supondría una pérdida irreversible de la biodiversidad, no sólo de la propia liana que nos ocupa, sino también los microorganismos asociados a la misma. El objetivo fundamental evaluar la biodiversidad de microorganismos de interés biotecnológico asociadas a la uva de vides silvestres y proveer datos que refuercen la importancia de la protección de *Vitis vinifera* ssp. *Sylvestris* (Gmelin) Hegi "*in situ*". Además de aportar nuevas cepas de levaduras capaces de hacer frente a problemas comunes entre países dónde la vitivinicultura es una importante fuente económica y social.

2. Material y métodos

La recogida uvas de vid silvestre se llevó a cabo en diferentes muestreos por triplicado en las diferentes regiones de estudio (Tabla 1) durante los meses de Septiembre, Octubre y Noviembre (según latitud y maduración de la uva) en el periodo del 2015 al 2017 la experiencia nos indica que en sistemas abiertos (campo) es importante hacer el muestro a larga escala (mínimo 2 años) para minimizar la influencia de las condiciones climáticas que afectan a las poblaciones de microorganismos presentes en la vid silvestre. Se emplearon bolsas asépticas para la recogida de bayas de uvas como muestra la metodología descrita por Cordero- Bueso et al. (2011). Se realizó un estudio ampelográfico para determinar que las uvas recogidas pertenecía a *V. vinifera* ssp. *Sylvestris* (Gmelin) Hegi siguiendo el protocolo de la O.I.V. También se llevaron a cabo fermentaciones a pequeña escala para aislar microorganismos al inicio, mitad y final del proceso fermentativo tal como se propone en Cordero-Bueso et al. [12]. Además, se aislaron los microorganismos directamente del hollejo de las uvas mediante lavados con cloruro sódico al 0,9% y con la ayuda de un sonicador y mediante rotura de las células vegetales por criogenización y con la ayuda de un bisturí para aislar levaduras endofíticas.

El aislamiento se realizó siguiendo protocolos de Microbiología Clásica. Los medios de cultivo utilizados fueron: medio YPD (2% glucosa, 2% peptona, 1% extracto de levadura, 2% agar) y medio WL-agar (Oxoid). Para su conservación y posteriores estudios de identificación y caracterización se utilizaron los siguientes métodos de conservación: a) en placas Petri, conservándose a 4ºC y b) en glicerinados (glicerol 40%), conservadas a –80ºC [12].

La identificación de los aislados se hizo mediante la obtención de los diferentes perfiles moleculares del ADN total y mitocondrial previamente extraído mediante protocolos estándares. Las cepas aisladas se identificaron por las técnicas de biología molecular PCR de la

Tabla 1. Puntos de muestreo de uvas de vid silvestre en las diferentes localizaciones de la región Euroasiática.

País	Localización	Provincia	Coordenadas (Latitud, Longitud)	Altitud (m)
Azerbayán	Guruchay 1	Quba	41.38564 N, 48.38264 E	953
	Guruchay 2	Quba	41.32566 N, 48.37580 E	880
Georgia	Tsminda, Gveleti	Gori	42.66244 N, 44.62045 E	1100
	Tsitsamuri, Mtsjeta	Kartli	41.52383 N, 44.43574 E	524
	Zhinvali 03, Dusheti 03	Kartli	42.08784 N, 44.45843 E	986
	Shirikhevi 06, Dusheti	Kartli	41.98121 N, 44.72292 E	978
	Nakhiduri 24, Marnueli	Kartli	41.29143 N, 44.41201 E	429
	Shirikhevi 02, Dusheti	Kartli	41.57850 N, 44.43076 E	1348
	Nakhiduri23, Marneuli	Kartli	41.29140 N, 44.41206 E	437
	Bagichala03, Dusheti	Kartli	42.02226 N, 44.44558 E	644
	Barisakmos, Gadasakmuevi 01	Dusheti	42.08769 N, 44.45849 E	738
Italia	Monte Fenera, Borgosesia	Vercelli	45.71000 N, 08.31600 E	886
	Montalto	Pavia	44.97940 N, 09.20941 E	381
	Ortuabis, Laconi	Oristano	39.85120 N, 09.05184 E	504
	Bau Sa Mela, Nurallao	Cagliari	39.83981 N, 09.12157 E	735
	Santa Sofia, Laconi	Oristano	39.85948 N, 09.12417 E	783
	Ristalu, Aritzo	Nuoro	39.95258 N, 09.19497 E	816
	Fluminimaggiore Nera	Carbonia-Iglesias	39.43689 N, 08.49750 E	62
	Fluminimaggiore Bianca	Carbonia-Iglesias	39.43689 N, 08.48871 E	71
	Gutturu Mannu 1	Cagliari	39.17276 N, 08.91511 E	116
	Gutturu Mannu 2	Cagliari	39.17276 N, 08.90545 E	116
Rumania	Turcul river, Bran	Brasov	45.51731 N, 25.36404 E	763
España	Hueznar, San Nicolás del Puerto	Seville	37.99448 N, 05.66693 W	565
	La Rocina, Doñana	Huelva	37.12380 N, 06.49654 W	8
	El Bosque, Benamahoma	Cádiz	36.77323 N, 05.48759 W	419
	Algeciras, Gran Capitán	Cádiz	36.12112 N, 05.52705 W	406
	La Algaida, Sanlúcar de Barrameda	Cádiz	36.83204 N, 06.31994 W	4
	Guadalupe	Cáceres	39.45132 N, 05.32724 W	637
	Tui	Pontevedra	42.04915 N, 08.64661 W	41

región ITS del ADN ribosómico y RFLP utilizando las endonucleasas de restricción *HaeIII, HinfI y CfoI* [12].

En el caso de las levaduras identificadas como *Saccharomyces cerevisiae*, se aplicó la técnica del análisis de los microsatélites multiplex o SSR con el objetivo de encontrar distintos perfiles a nivel de cepa [12]. Los amplificados se sometieron a electroforesis en geles de agarosa o en secuenciador automático. Y el análisis de las imagenénes en una cámara equipada con un transiluminador UV (BioRad). Se seleccionaron aquéllas que presentaron un perfil distinto y se tomaron al menos dos representantes de la misma especie para ser secuenciadas.

La secuenciación se llevó a cabo a través de los servicios de secuenciación MACROGEN Inc. Korea (`http://www.macrogen.com`). La alineación de sencuencias se realizó con programas de bioinformática online (CLUSTAL W, In-sillico, etc.). Las secuencias fueron comprobadas y depositadas en bases de datos electrónicas de colecciones de microorganismos (CECT, GenBank, NBCI, etc.).

Una vez bien identificados mediante las técnicas señaladas anteriormente, los microorganismos con diferentes perfiles moleculares fueron sometidos caracterización mediante pruebas bioquímicas y físico- químicas para conocer su capacidad de asimilación y fermentación de azúcares, resistencia al etanol y temperatura (curvas de crecimiento en lector de microplaca y fenotipo en placa medio-agar), al anhídrido sulfuroso, stress osmótico (curvas de crecimiento en lector de microplacas) y capacidad enzimática, se atenderá especialmente a las actividades glucosidasa, esterasa, proteasa y pectinasa (uso de kits enzimáticos y medios de cultivos específicos), ya que son de interés en la enología por aportar complejidad a los vinos. Se siguieron protocolos similares a los descritos en Rodríguez et al. [13]. También se obtuvieron las curvas de crecimiento de las cepas de levaduras de géneros *Saccharomyces* y con propiedades enológicas de interés mediante el uso un lector de placas multipocillos (96 pocillos) en mosto estéril natural obtenido a partir de vides silvestres, mosto obtenido de la variedad tempranillo, mosto sintético y en medio YPD como control durante 72 horas a 28ºC y obteniendo medidas de lectura de concentración de células por mililitro a una Densidad Óptica de 600 nm e inoculando a un O.D = 0,2–0,4. Una vez recogidos los datos se trataron en Excel para obtener las curvas de crecimiento y se ajustaron de acuerdo al modelo de de cinética de Gompertz descrito por Buchanan et al. [14]. Estas curvas de crecimiento nos proporcionaron información para determinar el momento exacto (horas de crecimiento) de la fase exponencial de estas cepas y conocer el comportamiento en mosto natural de *V. vinifiera* ssp. *Sylvestris* al que están adaptadas, de este modo conoceremos el momento óptimo de inoculación de las cepas posteriores experimentos de fermentación.

Los datos obtenidos en todos los objetivos se analizaron a través de XSTAT. Además, se usaron programas específicos para los análisis filogenético (PAUP v4b10 y Modeltest 3.06), análisis Bayesianos (MRBAYES v3.0b4), análisis de parsimonia heurística y árboles filogenéticos (MAXTREES, PAUP v4b10).

3. Resultados y discusión

El número de cepas muestreadas durante el periodo 2013–2016 asciende a 29 puntos de muestreo (2 en Azerbaijan, 9 en Georgia, 10 en Italia, 1 en Rumanía y 7 en España) en los cuales al menos se tomaron muestras de uva de entre 3 y 5 cepas de vid silvestre. La cantidad de uvas recolectadas fue variable, entre 100 g y 2,5 kg. El grado de maduración de la uva siempre fue óptimo para poder llevar a cabo microvinificaciones como método de autoenriquecimiento con objeto de aislar el número máximo de especies. De todas la muestras tomadas y analizadas se obtuvo un total de 3180 colonias de las cuales, una vez identificadas mediante los métodos moleculares mencionados en la sección de Materiales y Métodos se identificó un total de 50 especies pertenecientes a 10 géneros. La Tabla 2 muestra la frecuencia y distribución de las especies identificadas en la región Euroasiática. Entre los aislados, 5 cepas no fueron identificadas mediante los métodos moleculares conocidos hasta ahora, no obstante fueron caracterizados mediante técnicas clásicas de microscopía y a través de pruebas bioquímicas de fermentación y asimilación de azúcares (datos no mostrados). Adicionalemente, se aislaron levaduras viables pero no cultivables e igualmemente fueron caracterizadas siguiendo los procedimientos mencionados anteriormente. La especie mayoritaria aislada fue *Saccharomyces cerevisiae*, esto es debido al procedimiento experimental empleado de autoenriquecimiento, no obstante, esta especie también fue aislada directamente del hollejo de las uvas recolectadas, aunque en una baja proporción. Todos los aislados pertenecientes al género *Saccharomyces* fueron sometidos a posteriores análisis específicos para obtener diferentes perfiles a nivel de cepa (genotipos). Respecto a levaduras de géneros No-*Saccharomyces*, las especies *Hanseniaspora uvarum* y *Pichia kluyvery* fueron las especies mayoritarias, pero no se aislaron en todas las regiones muestreadas (Tabla 2). Algunas especies fueron exclusivas de cada área, como por ejemplo *Aureobasidium proteae* que solamente fue aislada en Italia, al igual que sucede con especies de los géneros *Cryptococcus*, *Curvibasidium* y algunas de *Hanseniaspora*. Las especies *Clavispora lusitaniae*, *Hanseniaspora meyeri*, *Issatchenkia terricola* y *Xanthophyllomyces dendrorhou* solo se aislaron en Georgia, al igual que sucede con *Saccharomycodes ludwigii* en Azerbayán. Rumanía solo mostró una especie exclusiva entre los aislados, *Pichia fermentans*. En el caso de España cabe descatar que fue la región donde más especies diferentes se aislaron, podría venir motivado por haber muestreado un año más que en el resto de países, sin embargo, también se identificaron numerosas especies no aisladas en otras áreas del Mediterráneo tales como *Candida ethanolica*, *Candida sake*, *Candida stellata*, *Candida zemplinina*, *Lachancea thermotolerans*, *Meyerozyma caribbica*, *Pichia kudriavzevii*, *Rhodosporidium palidugenum*, *Rhodotorula glutinis*, *Scheffersomyces stipitis*, *Schwanniomyces polymorphus*, *Torulaspora delbrueckii* y *Wickerhamomyces anomalus*. Pese a que ninguna de las especies identificadas en los diferentes países donde se realizó el muestreo aparece en todos los países, a excepción de *Saccharomyces cerevisiae*, cabe destacar que la especie *Pichia manshurica* fue aislada en Italia y España pero a la misma latitud (Monte Fenera y Tui).

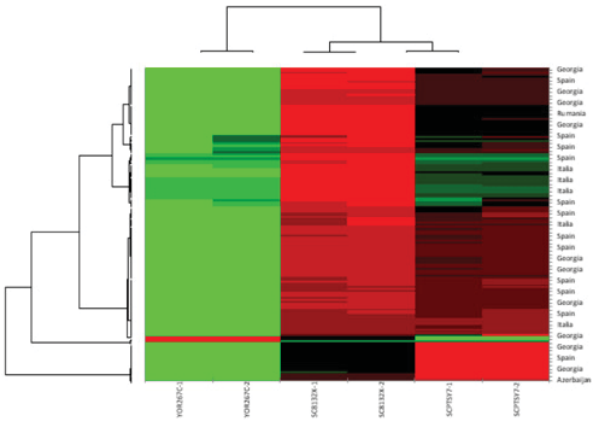

Figura 1. Mapa de calor (Heatmap) de los diferentes genotipos obtenidos mediante PCR-Multiplex de Microsatélites en los diferentes países de la región Euroasiática muestreados.

Aquellos aislados que fueron identificados como pertenecientes al género *Saccharomyces*, dado el interés que suscita en la industria enológica, fueron posteriormente sometidos análisis de PCR Multiplex de microsatélites. Entre los aislados de S. cerevisiae se obtuvieron un total de 169 genotipos diferentes, siendo 80 de España, 56 de Georgia, 24 de Italia, 6 de Azerbaijan y 3 de Rumanía. Se observó cierta relación entre los genotipos de todas la regiones pero sólo para algunos alelos. A continuación se explican los resultados obtenidos.

En la Fig. 1 se muestra el de mapa de calor obtenido a partir del análisis genómico (en base al tamaño en pares de bases del amplificado) por PCR multiplex de microsatélites con las tres parejas de primers. Los alelos se agrupan en filas y las cepas en columnas. Si se observamos individualmente los alelos y las cepas según su origen (Azerbayán, Georgia, Italia, Rumanía y España) vemos claramente que los se dividen en tres grupos que corresponden a los tres genes amplificados.

Según el cluster de la izquierda (dendrograma) se observa que las diferentes cepas de *S. cerevisiae* aisladas de uvas y microvinificaciones de uvas silvestres recogidas en las 5 áreas estudiadas se agrupan de acuerdo al tamaño de los alelos amplificados.

Si nos centrarnos en los patrones rectángulo/cuadrado dentro del mapa, los rectángulos grandes verdes de la izquierda muestran que para los alelos (YOR267) tenemos una coincidencia relativamente alta entre las cepas aisladas en los diferentes países, fijándonos en la parte central del mapa se muestra un rectángulo rojo grande que aúna coincidencias entre los alelos (SC8132) de los diferentes países, pero con una menor frecuencia que en el primer patrón y además se alejan bastante los aislados en Azerbayán (restángulo marrón oscuro) del resto de países. En el caso del tercer patrón de colores más oscuros (marrón-negro) es completamente inverso, en comparación con los dos anteriores, así como el rectándulo verde intenso correspondiente a algunos genotipos aislados en Georgia demuestran la variabilidad y exclusividad de algunos genotipos al área Caucásica. Curiosamente se observa que algunos genotipos encontrados en España comparten varios alelos con aquéllos de los países caucásicos (cuadros verdes, negro y rojo del de la parte inferior del mapa de calor) agrupados en un cuarto cluster (dendrograma de la izquierda). Con estos resultados se

Especies	Azerbayán	Georgia	Italia	Rumanía	España
Aureobasidium proteae			2		
Aureobasidium pullulans			7		18
Candida ethanolica					1
Candida sake					11
Candida stellata					25
Candida zemplinina					4
Clavispora lusitaniae		32			
Cryptococcus flavescens			9		
Cryptococcus wieringae			10		
Curvibasidium cygneicollum			2		
Curvibasidium pallidocorallinum			15		
Hanseniaspora sp			2		
Hanseniaspora clermontiae			9		
Hanseniaspora guilliermondii			18		54
Hanseniaspora meyeri		13			
Hanseniaspora opuntiae			32		120
Hanseniaspora osmophila					
Haseniaspora uvarum		67	158	34	137
Hyphopichia pseudoburtonii			8		
Issatchenkia siamensis		27	5		
Issatchenkia terricola		17			
Lachancea thermotolerans					185
Martiniozyma asiatica			13		
Metschnikowia sp.		21	15		
Metschnikowia fructicola			69	15	
Metschnikowia pulcherrima			36		19
Metschnicowia viticola		18	23		
Meyerozyma caribbica					88
Meyerozyma guilliermondii			30		107
Naganishia diffluens			9		
Pichia fermentans				10	
Pichia kluyveri			43		230
Pichia kudriavzevii					130
Pichia Manshurica			4		24
Rhodosporidium paludigenum					4
Rhodotorula fujisanensis			21		
Rhodotorula glutinis					34
Rhodotorula mucilaginosa		34	1		22
Rhodotorula nothofagi			27		
Saccharomyces cerevisiae	50	296	249	31	517
Saccharomycodes ludwigii	10				
Scheffersomyces stipitis					14
Schwanniomyces polymorphus					2
Torulaspora delbrueckii					67
Wickerhamomyces anomalus					42
Xanthophyllomyces dendrorhou		2			
Zygosaccharomyces sp			2		
Zygosaccharomyces bailii					6
Zygosaccharomyces fermentati					41
Zygosaccharomyces rouxii			2		6
Viables pero no cultivables			30		
No identificada		3			2

podría afirmar que existen tres regiones, áreas o ejes definidos en cuanto a genotipos de levaduras, serían el eje oriental (Azerbaijan y Georgia), el eje central (Rumanía e Italia) y el eje occidental (España, pese a que comparte algunos genotipos con el eje oriental).

4. Conclusiones

Este estudio destaca el potencial de biodiversidad de entornos prístinos que aún representan una fuente fascinante para enfrentar problemas comunes en la elaboración del vino. Se están llevando a cabo estudios de evaluación del potencial biotecnológico de las diferentes especies aisladas, así como estudios para determinar si las cepas no identificadas son posibles nuevas especies.

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Wild Grape-Associated Yeasts as Promising Biocontrol Agents against *Vitis vinifera* Fungal Pathogens

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The increasing level of hazardous residues in the environment and food chains has led the European Union to restrict the use of chemical fungicides. Thus, exploiting new natural antagonistic microorganisms against fungal diseases could serve the agricultural production to reduce pre- and post-harvest losses, to boost safer practices for workers and to protect the consumers' health. The main aim of this work was to evaluate the antagonistic potential of epiphytic yeasts against *Botrytis cinerea*, *Aspergillus carbonarius,* and *Penicillium expansum* pathogen species. In particular, yeast isolation was carried out from grape berries of *Vitis vinifera* ssp sylvestris populations, of the Eurasian area, and *V. vinifera* ssp *vinifera* cultivars from three different farming systems (organic, biodynamic, and conventional). Strains able to inhibit or slow the growth of pathogens were selected by *in vitro* and *in vivo* experiments. The most effective antagonist yeast strains were subsequently assayed for their capability to colonize the grape berries. Finally, possible modes of action, such as nutrients and space competition, iron depletion, cell wall degrading enzymes, diffusible and volatile antimicrobial compounds, and biofilm formation, were investigated as well. Two hundred and thirty-one yeast strains belonging to 26 different species were isolated; 20 of them, ascribed to eight species, showed antagonistic action against all molds. Yeasts isolated from *V. vinifera* ssp sylvestris were more effective (up to 50%) against *B. cinerea* rather than those isolated from *V. vinifera* ssp vinifera. Six strains, all isolated from wild vines, belonging to four species (*Meyerozyma guilliermondii*, *Hanseniaspora uvarum*, *Hanseniaspora clermontiae,* and *Pichia kluyveri*) revealed one or more phenotypical characteristics associated to the analyzed modes of antagonistic action.

Keywords: yeasts, molds, *V. vinifera* ssp sylvestris, biocontrol, fungal diseases

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INTRODUCTION

Plants provide over 80% of the human diet. Just three cereal crops (i.e., rice, maize, and wheat) and two fruit crops (grape-berries and citrus fruits) provide 70% of energy intake and cope the production of 80% of the fermented beverages in the world (FAO, 2011). Since the 1900s, around 75% of crop diversity has been lost from farmers' fields. Regarding harvest products, many losses (up to 25% of total production in industrialized

countries and more than 50% in developing countries) are attributed to decay fungi, such as the *Botrytis*, *Penicillium, Aspergillus,* or *Cholletotrichum* genera, which are also the source of mycotoxins, harmful compounds to humans (FAO, 2011). The control of fungal diseases and mycotoxins in food and feed chains is principally based on the use of synthetic fungicides. In 2015, Spain, France, Italy, and Germany together made up 70.5% of the European Union-28's pesticide sales. Fungicides are also increasing the level of hazardous residues in the environment, they are becoming less effective due to both the increasing of resistant fungal strains, and the use of restrictions carried out by the European authorities (Directive 2009/128 /EC). Natural diversity and ecosystems provide agricultural production in many different ways (Power, 2010), but not all are well-known. Although animal and plants have received considerable attention as a resource for natural-product discovery, the microbiological component of this natural richness remains relatively unexplored.

Yeasts are unicellular fungi that have been isolated from different ecosystems and sources both natural and in connection with human activities. They can be found on/in fruits, including *Vitis vinifera* ssp vinifera cultivars and *V. vinifera* ssp. sylvestris, plants, insects, animal intestinal tracts, soils, and marine environments (Kurtzman et al., 2011). In the past 35 years, there have been extensive research activities to explore and develop the potential of yeasts as antagonists to biologically control harvest pathogens and as an alternative to chemical pesticides (Liu et al., 2013). Representing an eco-friendly alternative to synthetic pesticides, the use of antagonist yeasts as biocontrol agents has generated a great enthusiasm (Wisnieswski et al., 2007; Droby et al., 2009; Sipiczki, 2016; Spadaro and Droby, 2016). However, yeasts often show a lower and non-comparable effectiveness against pathogenic fungi (*Botrytis cinerea, Aspergillus carbonarius, and Penicillium expansum*) in comparison to chemical fungicides (Liu et al., 2013), thus reducing their practical applications and leaving the problem of plant fungal disease still unsolved. Considerable progress has been made in increasing knowledge and commitment to elucidate some modes of action of few yeast strains against pathogenic fungi (Sipiczki, 2006; Sharma et al., 2009; Jamalizadeh et al., 2011; Spadaro and Droby, 2016). The described mechanisms are; nutrient or space competition (Suzzi et al., 1995), iron depletion (Sipiczki, 2006; Parafati et al., 2015), extracellular lytic enzymes production (Bar-Shimon et al., 2004), volatile organic compounds (Fredlund et al., 2004), reactive oxygen species (ROS) tolerance (Jamalizadeh et al., 2011; Liu et al., 2011), biofilm formation (Giobbe et al., 2007; Wisnieswski et al., 2007), or inducing host-plant resistance throughout the accumulation of phytoalexins (Arras, 1996; Jeandet et al., 2002) and the synthesis of pathogenesis-related proteins (Chan and Tian, 2006). Inhibition capabilities on mycelial growth or conidia germination in molds have been reported by some yeast strains of species living in vineyards, overwintering grapes, and cellar ecosystems (Elmer and Reglinski, 2006; Nally et al., 2012; Sipizcki, 2016). Nevertheless, all the scientific strategies focused on looking at different components of such interactions separately or taking into consideration binary or ternary trophic levels of the host-pathogen-antagonist interplay (Droby et al., 2009; Spadaro and Droby, 2016). In general, interactions are not between two single microorganisms and the host; they also involve the native microbiota of the host and the environmental factors (i.e., the variation of the climatic conditions and other abiotic factors such as the soil, plant emplacement, or nutrient availability for the plant). In the case of the vineyards, efforts to understand the influence of different agronomic parameters on yeast populations associated to grape-berries have been published (Cordero-Bueso et al., 2011a,b, 2014) but there is still a lack of bibliography. Moreover, there are unexplored ecosystems such as wild vines like the protected species *V. vinifera* ssp sylvestris (Gmelin) Hegi which could represent a great reservoir of novel and promising yeast species to be used in the food industry, as well as a substitutive of agrochemicals.

The main aim of this work was to evaluate the antagonistic potential of yeasts isolated from grape berries collected from *V. vinifera* ssp sylvestris populations in the Mediterranean and Black Sea basins and from *V. vinifera* ssp vinifera cultivars managed under three different farming systems: organic, biodynamic, and conventional. The mode of action and the grape-berry population associate to grape-berries were investigated as well.

MATERIALS AND METHODS

Yeast Strain Identification

Yeast strains were isolated between 2013 and 2016 from grape berries collected in Georgia, Italy, Romania, and Spain from *V. vinifera* ssp. sylvestris populations as stated in Cordero-Bueso et al. (2017) and in Italy from *V. vinifera* ssp. vinifera cv. Pinot Noir cultivated in three different farming systems: organic, biodynamic, and conventional in 2014 (**Figure 1**). Grape samples were treated following the protocol of Vigentini et al. (2016). All yeasts used in this work were stored in YPD medium (20 g/L peptone, 10 g/L yeast extract, 20 g/L glucose) added with 20% (v/v) glycerol at −80°C. Fresh yeast cultures were obtained by inoculation 1% (v/v) glycerol stocks in YPD broth at 25°C for 3 days in aerobic conditions. Isolates were also plated onto Wallerstein Laboratory Nutrient Agar (WL) to evaluate colony diversity as suggested by Pallmann et al. (2001). DNA extraction from the yeast isolates was performed according to Querol et al. (1992). The patterns belonging to the different species were obtained by Restriction Fragment Length Polymorphism (RFLP) analysis of the amplified ITS1-5.8S-ITS2 region; the primers used for DNA amplification were ITSY1 (5′-TCCGTAGGTGAACCTGCGG-3′) e ITSY4 (5′-TCCTCCGCTTATTGATATGC-3′) as described by White et al. (1990). PCR products were digested by *Cfo*I, *Dde*I, *Hae*III, and *Hinf*I restriction enzymes (Thermo Fisher Scientific, Massachusetts, U.S.A.). *Meyerozyma guilliermondii* (anamorph *Candida guilliermondii*) and *Meyerozyma caribbica* (anamorph *Candida fermentati*) are closely related species. Thus, to avoid misidentification these species of yeasts were also subjected to RFLP analysis using the enzyme *Taq*I as stated by Romi et al. (2014). Amplification products and their fragments were separated on 1.4% (w/v) and 2.5% agarose gel, respectively, added with 0.05 μg/L of ethidium bromide in TAE buffer

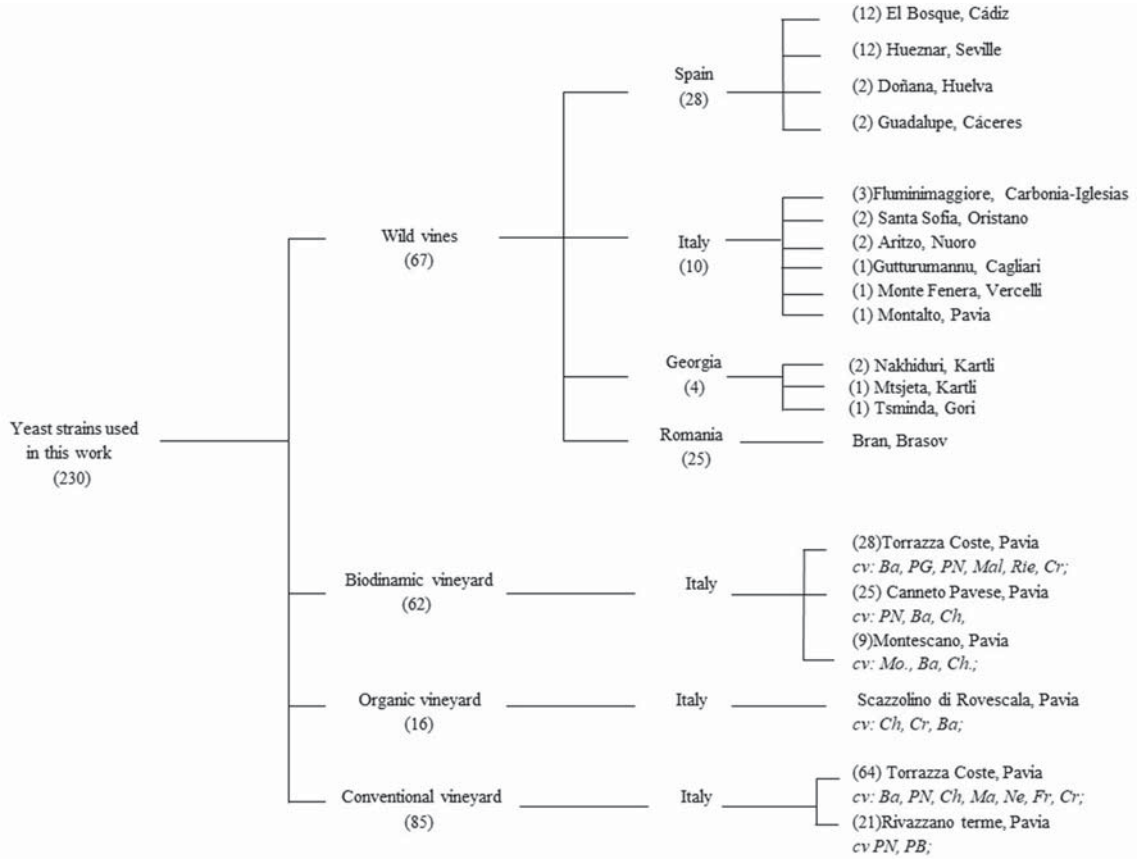

FIGURE 1 | Origin and source of the yeast strains assayed in this work.

(Tris-acetate 40 mM, EDTA 1 mM, pH 8) at 100 V for 90 min. The agarose gels were visualized using UV and photographed (1000 System, Bio-Rad Laboratories, California, U.S.A.). At least two representative members from each ITS-RFLP genotype group were randomly selected for sequencing LSU sRNA gene D1/D2 domain. Certain database sequences of several species such as *Aureobasidium pullulans* and *Rhodotorula nothogafi*, have identical D1/D1 sequences with other species. Thus, when necessary, we included the ITS1-5.8S-ITS2 region sequences. Amplification of D1/D2 region was carried out using primers NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3), as previously described Kurtzman and Robnett (1998). Purification and sequencing of PCR products were performed by Macrogen Inc. facilities (Seoul, South Korea) using an ABI3730 XL automatic DNA Analyzer. The obtained sequences were aligned using ClustalX algorithm. The Basic Local Alignment Search Tool (BLAST) (http://www.ebi.ac.uk/blastall/nucleotide.html) was used to compare the sequences obtained with databases from the European Molecular Biology Laboratory (EMBL). As proposed Sipiczki (2016), the sequences of the strain types were also determined by pairwise Blast alignment using the bl2seq algorithm available at the website of the NCBI (http://www.cbs.knaw.nl). We considered an identification as "correct" when the gene sequence showed an identity $\geq$ 98% and a good query cover with the exception of the species *Vishniacozyma carnescens* and *V. victoriae* which D1/D2 sequences of their type strains differ only by 1.8%. Moreover, yeast strains were tested for the fermentation or assimilation of the different compounds as sole carbon, nitrogen, and others sources, with the exception of the hexadecane, vitamin-free, 5-keto-D-glucanase, saccharate, cadaverine, and CoQ component, as stated in Kurtzman et al. (2011) but using a 96-well microtiter plate technology.

Mold Strains and Growth Conditions

The mold strains used in this work were *P. expansum* UCAF0034 (Colección de la Universidad de Cádiz, Spain), *B. cinerea* BO5.10 (*Colección Española de Cultivos Tipo,* Burjassot, Valencia, Spain), and *A. carbonarius* UCAF0012 (Colección de la Universidad de Cádiz, Spain). Molds were selected based on their virulence by artificial inoculation on wounded grapes (data not shown).Mold cultures were plated on a Potato Dextrose Agar medium (Conda Laboratories, Torrejón de Ardoz, Madrid, Spain). Plates were incubated at 25°C under constant white light for at least 10 days. After incubation, spores were collected in a solution of 0.1% (v/v), Tween 20 (SIGMA). The concentration of the conidial suspension was adjusted to give 6×10^6 spores/mL according

to Comménil et al. (1999). Mold strains were stored as conidial suspensions added with 20% (v/v) glycerol at −80°C.

In Vitro Assays for Antagonistic Activity

Dual Screening of Antagonistic Activity on Agar Media

The antagonistic activity of the 241 yeast isolates against *A. carbonarius, B. cinerea,* and *P. expansum* molds was investigated by *in vitro* assay. In the *first* screening, 5 μL of a fresh conidial suspension of the molds, one for each plate, were inoculated in the center of the PDA plate. Then, 5 μL of six fresh yeast cultures were positioned at 2.5 cm from the center of each Petri dish. The plates were incubated at 25°C for 10 days under constant white light and 80% relative humidity. A clear zone around the yeast colonies was interpreted as total inhibition of the growth of the mold. The strains showing an inhibitory activity were chosen for the second step of selection. In this case the PDA plates were prepared as follows: 10 mL of PDA were first included in each plate; afterwards, 5 mL of soft PDA (7 g/L agar) containing a final concentration 10^6 CFU/mL of yeast cells, one for each strain, were inoculated in the plates. Subsequently, when the plates were solidified, 5 μL of fresh conidial suspensions of the tested molds were inoculated upon them. The plates were incubated at the same conditions of first screening. After incubation, the radial growth was measured and the inhibition percentage was calculated as follows: inhibition (%) = (DC – DA)/DC x 100, where DC is the diameter of the growth area without the antagonistic yeast (control), DA is the diameter of growth area with the antagonistic yeast (Ruiz-Moyano et al., 2016). The experiments were repeated three times to confirm reproducibility of the results.

Evaluation of the Minimum Inhibiting Concentration

An estimation of the starting concentration of yeast cells capable to inhibit the mold growth was carried out by the following test. Fresh cultures of the yeasts that overcome the second step of selection were grown in YPD broth at 25°C for 3 days. PDA plates were prepared for each strain containing a different cell concentration, from 10^3 to 10^6 CFU/mL. When the plates solidified, 10 μL of conidial suspensions (3×10^5 spores/mL) of *B. cinerea*, *A. carbonarius,* and *P. expansum* were spotted on the center of the Petri dish. The plates were incubated at 25°C for a week under constant light. The results were considered positive when the yeast was able to inhibit the total mold growth within the time of incubation. Control tests without inoculated yeast cells were carried out. The experiments were repeated three times to confirm reproducibility of the results.

Killer Character Assay

The killer character assay was performed according to Stumm et al. (1977). Plates containing YPD-agar and 0.003% (w/v) of methylene blue that was buffered to pH 4.5 with 0.1 mol/L of citrate-phosphate buffer were used. Yeast strains were cultured in liquid YPD until their exponential growth phase. Then, yeast strains were diluted in YPD and spread onto the plates at a concentration of 10^5 cells per plate and incubated at 25°C for 48–96 h. Killer activity was scored positive when the killer strain was surrounded by a region of bluish-stained cells, or by a clear zone of growth inhibition bounded by stained cells.

Test for Lytic Enzymes Activity

In order to investigate the reason of the observed inhibitory effect, the previous selected strains were examined taking in consideration the production of cell wall lytic enzymes. Yeast fresh cultures were adjusted at a final concentration of 1×10^6 CFU/mL. To evaluate the proteolytic activity, 20 μL of the yeast suspension were spotted onto Skim Milk agar (Merck, Darmstadt, Germany); the formation of a clear halo around the colony after incubation at 25°C for 5 days indicated the enzymatic activity. Glucanase and chitinase activities were determined by replica plating technique. In this case, 20 μL of the yeast suspension were spotted onto YPD plates containing 0.2% β-glucan (Sigma, Town, Nation) and YPD plates containing 0.2% chitin (Sigma). Petri dishes were incubated at 30°C for 5 days. Colonies were rinsed off the plates with distilled water before staining the plates with 0.03% (w/v) Congo Red. A clear zone around the colony meant the presence of glucanase activity. Yeasts were screened for polygalacturonase production with the method described by Strauss et al. (2001) as well; they were spotted onto polygalacturonate Agar Medium containing 12.5 g/L polygalacturonic acid (Sigma), 6.8 g/L potassium phosphate (pH 3.5), 6.7 g/L yeast nitrogen base without ammonium sulfate (YNB, Difco), 10 g/L glucose, and 20 g/L agar. Plates were incubated at 30°C for 5 days. Colonies were rinsed off the plates with deionized water before staining the plates with 0.1% (w/v) Ruthenium Red. Colonies showing a purple halo were considered positive. β-glucosidase activity was tested by plating the yeast onto a selective medium containing 6.7 g/L yeast nitrogen base (YNB, Difco), 5 g/L arbutin (Sigma), and 20 g/L agar (pH 5.0). Two milliliters of a filter-sterilized 1% (v/v) ammonium ferric citrate solution was added to 100 mL media before pouring onto the plates. Petri dishes were incubated at 30°C for 3 days. Positive colonies were identified by the discoloration of the media to a brown color.

Production of Volatile Organic Compounds (VOCs) and Hydrogen Sulfide Release

Selected yeast strains were also evaluated for their production of VOCs and hydrogen sulfide released against the molds *B. cinerea, A. carbonarius,* and *P. expansum*. Four-part Petri dishes containing 3.5 mL of PDA for each sector were used. In one part, 20 μL of 10^6 CFU/mL of yeast suspension were inoculated. The plates were incubated at 25°C for 3 days. Then, 20 μL of conidial suspension (6×10^6 spores/mL) of each mold were inoculated in the other three sectors of each plate. Plates without the inoculation of yeasts were utilized as control. Finally, the plates were double wrapped with sterile HDPE film (Parafilm, Neenah, U.S.A) to prevent air escape and incubated for 3 days at 25°C under constant white light. Radial growth reduction, in relation to the control test, was calculated after 6 days. All experiments were performed in triplicate. Data were analyzed by one-way ANOVA. The means were separated at the 5% significance level using Tukey's test. The yeast strains slowed or inhibited the

mold growth were also tested for the production of acetic acid and hydrogen sulfide. Ten microliters of yeast cell suspensions (10^6 CFU/mL) were spotted on Biggy Agar (Oxoid, Bakingstoke, U.K.) and in a $CaCO_3$ agar medium (5.0 g/L yeast extract; 20 g/L glucose; 10 g/L $CaCO_3$; 20 g/L agar). The plates were incubated at 30°C for 3 days. The qualitative amount of H_2S production on this indicator medium was determined by the color of the colonies, which ranged from white (no release) through brown to near black, depending on the extent of production (high release). In the case of the acetic acid production, a clear zone around the colony meant the presence of acetic acid. A halo greater than 3 mm of radius meant a high acid release, if the halo was between 2 and 3 mm meant low acid release, if the halo was between 1 and 2 mm meant slight acid formation, and if the halo was less than 1 mm meant traces.

Biofilm Formation

The capability to produce biofilm was evaluated following the protocol of Jin et al. (2003) partially modified. Ten microliters of fresh yeast suspension as previously described were inoculated in 1 mL of Yeast Nitrogen Base (YNB, Difco, Swedesboro, U.S.A.) added with 100 mM glucose and incubated overnight at 28°C. Subsequently, the tubes were centrifuged at 4,000 rpm for 5 min (Rotina 380 R, Hettich Zentrifugen, Tuttlingen, Germany), the cells were washed twice with a 1X phosphate-buffered saline (10X PBS: NaCl 1.37 M, KCl 27 mM, Na_2HPO_4 100 mM, KH_2PO_4 18 mM), pH 7.2) and re-suspended in YNB + glucose (100 mM) medium to obtain 10^7 CFU/mL. A control test was prepared with the medium without yeast cells added. One hundred microliters of the cell suspension were inoculated in triplicate into 96-well polystyrene plate with flat bottom (Starlab, Hamburg, Germany) at 28°C in a shaker at 75 rpm for 3 h. After the adhesion phase, the wells were washed twice with 150 μL of PBS, and then 100 μL of same medium were added into each well and incubated at 28°C in a shaker at 75 rpm for 72 h. The medium was sucked up daily and, then, 100 μL of fresh YNB were put into each well. After incubation, the wells were washed twice with 150 μL of PBS then 100 μL of crystal violet 0.4% (w/v) were put into each well. After 45 min, the wells were washed again for four times with 150 μL of distillate sterile water and immediately 200 μL of 95% (v/v) ethanol were added. After 45 min, 100 μL of solution were transferred to a new polystyrene 96-well plate and then the solution was measured at 590 nm. The absorbance values were subtracted for the control test values.

Effect of Iron Concentration on the Inhibitory Activity of the Yeast Strains

In order to investigate the influence of iron concentration on the inhibitory activity of the selected yeasts the following test was carried out. PDA plates without added iron and plates with 5 and 20 μg/mL of $FeCl_3$ were prepared spreading on plates a conidial suspension (3×10^5 spores/mL) of *B. cinerea, A. carbonarius,* and *P. expansum*. Then, 10 μL of yeast suspensions (10^6 CFU/mL) were dropped on Petri dishes in triplicate. Three plates for each mold without yeast addition were used as control. The plates were incubated at 25°C for 1 week under constant white light. The width of reddish halos developing around the yeast colonies were measured according to Parafati et al. (2015). The results of the role of competition for iron on the antagonistic activity of the yeasts were obtained measuring the width of inhibition zones around the yeast colonies after a week.

Effect of Other Metabolites Released by Yeast Strains on Mold Growth

In order to examine the effect of other potential metabolites derived from the primary or secondary metabolism of yeasts produced by antagonistic yeasts, the molds were grown in a medium containing the supernatant of a yeast culture. The yeast cultures were grown in 50 mL YPD broth at 25°C for 5–7 days in a shaker at 125 rpm. The cell growth was monitored by spectrophotometer measurements at 600 nm (Jenway 7315, Staffordshire, U.K.). When yeast cultures attained the stationary phase the supernatants were collected by centrifugation at 3,500 rpm for 5 min at 4°C (Rotina 380 R, Hettich Zentrifugen, Tuttlingen, Germany) and filtered by a 0.45 μm sterile membrane (Minisart, Goetting, Germany). Five, 0.5, and 0.05 mL of supernatants were mixed with warm (<45°C) and concentrated 5X PDA medium by adjusting the volume with sterile distilled water and poured in Petri dishes. When the plates solidified, 10 μL of conidial suspensions (3×10^5 spores/mL) of *B. cinerea*, *A. carbonarius,* and *P. expansum* were inoculated. The plates were incubated at 25°C for a week under constant light. The test was considered positive if the tested molds did not grow or if a severe growth inhibition was observed with respect to the control.

In Vivo Assays for Inhibitory Activity

Efficacy of Yeast Strains in Controlling Grapes Infected by Molds

The yeast strains showing an evident inhibitory activity by *in vitro* assays were selected for the *in vivo* test. Fresh yeast cultures were collected by centrifugation at 3,000 rpm (Rotina 380 R, Hettich Zentrifugen, Tuttlingen, Germany) for 5 min at 4°C and washed twice with sterile distilled water. The yeast suspensions were adjusted at 10^6 CFU/mL. Healthy berries of table grapes (cultivar Superior Seedless, Egypt) were used for the test. Grape berries surface was disinfected by dipping them in a solution 1% (v/v) sodium hypochlorite for 5 min and rinsed three times with sterile distilled water. Afterwards, three berries for treatment were cut with a sterile scalpel (one wound of 5 mm for each berry) and submerged in the yeast cells suspensions for 5 min. The berries were put into sterile 50 mL Falcon tubes (Sigma-Aldrich, Darmstadt, Germany) and incubated for 24 h at 25°C. Then, the wounds were inoculated with 20 μL of conidial suspension (6×10^6 spores/mL) of *B. cinerea, A. carbonarius,* and *P. expansum* (three berries for each mold and for each yeast) and incubated at 25°C under constant light for a week. Three berries for each mold without yeast cells were used as control. The disease severity was evaluated by a visual score "1-to-4" (1: no visible symptoms; 2: soft rot; 3: formation of mycelium; 4: sporulation of mold) according to Parafati et al. (2015).

Inhibitory Effect of Yeasts vs. a Chemical Pesticide by *in Vivo* Tests

The inhibiting activity of strains, that showed the best results in the previous tests, were compared to the commercial pesticide Switch®, Syngenta (37.5% *Cyprodinil* and 25% *Fluodioxinil*). The fresh yeast cultures were prepared as above described. The pesticide was used at the suggested concentration of 1 g/L, according to the manufacturer's instruction, and it was dissolved in 25 mL of distilled sterile water. Healthy berries of table grape (cultivar Sugarone, Chile) for each yeast strain, pesticide, and control, repeated for the three tested molds, were used in this trial. The berries were treated and disinfected as above described. Afterwards, the berries were submerged in the solutions containing the yeast cells and in the solution containing the chemical pesticide for 5 min. Three berries for each mold without yeast cells and pesticide were used as control. The berries were included in six-well plate (Starlab, Hamburg, Germany) at 25°C for 24 h. Then, 10 μL of conidial suspension (6×10^6 spores/mL) of *B. cinerea*, *A. carbonarius,* and *P. expansum* were inoculated on the berries, in the correspondingwound points. The plates were incubated at 25°C for a week under constant light. The results were evaluated by a visual score previously stated.

RESULTS

Identification of Yeasts

Two hundred and thirty-one yeast strains were isolated from grape berries samples of different vines: 85, 62, and 16 from a conventional, a biodynamic, and an organic vineyard, respectively. Sixty-seven yeasts were collected from *V. vinifera* ssp. sylvestris. The sampling plan and the distribution of the isolates are reported in Supplementary Material 1. Sixteen different morphologies were observed on WL-agar plates (data not shown). Three distinct colony subtypes were also identified within the pink-halo producers. Molecular identification by using amplification and restriction analysis of ITS1-5.8S-ITS2 region revealed 26 different patterns. The D1/D2 region of the 26S rDNA gene of at least two yeast strains, for each potential species was sequenced to identify the species. **Table 1** shows the number of strains ascribed to each different species. The accession number of the sequences deposited at GenBank and the most similar CBS strain numbers are shown in **Tables 1**, **3**. *Aureobasidium pullullans* can easily be confused with *Aureobasidium subglaciale*, *Kabatiella microsticta,* or *Columnospaeria fagi* because many database sequences of these species have identical D1/D2 sequences (Brysch-Herzberg and Siedel, 2015; Sipiczki, 2016). Moreover, *R. nothofagi* is difficult to distinguish from *C. pallidicorallinum* because certain database of sequences of these species have identical D1/D2 sequences (Sampaio, 2011; Sipiczki, 2016). Therefore, we analyzed the ITS region of *A. pullulans* and *R. nothofagi* as well (**Table 1**). Since mating partners of the type strains of these species exhibited the most similar ITS sequences and the most similar D1/D2 sequences it's justified to assign the yeast strains of this study to *A. pullulans* and *R. nothofagi*. Furthermore, our strain of *R. nothofagi* did not grow on maltose, trehalose, and inulin, which are usually assimilates by *C. pallidicorallinum* (Sipiczki, 2016). The D1/D2 sequence of our strain identified as *V. carnescens* totally fits with the sequences of type strains found in the explored databases.

Unfortunately, we encountered the problem that isolates ROMA1A, ROM10, CABM7C, and CABM9C (**Table 1**) which seem to belong to *Metschnikowia*-like strains, did not show sequence identity of their D1/D2 to any of the type strains despite they were fairly similar to one species of the *Metschnikowia pulcherrima* clade. It happened also with the ITS sequences. In agreement with Lachance (2011), Sipiczki et al. (2013), Brysch-Herzberg and Siedel (2015), Lachance (2016), and Sipiczki (2016), species belonging to the *M. pulcherrima-like* strains cannot be unequivocally assigned to one of the species of this clade after rDNA analysis because some species such as *M. fructicola* or *Metschnikowia andauensis* have a non-homogenized rDNA array. Moreover, these yeast strains cannot be easily separated by phenotypical and physiological tests. Efforts to clarify the taxonomic situation of the *Metschnikowia* clade are required. Although was impossible to assign our strains to one of the currently described species in the *M. pulcherrima* group, we showed in **Tables 1, 3**, the most probable species related to this genus according to the results obtained after the analysis performed.

In Vitro Tests

In Vitro Dual Assays to Show the Antagonist Yeast-Mold Interactions

All yeast isolates were subjected to a preliminary *in vitro* assay for the detection of an antagonistic activity against *B. cinerea*, *P. expansum,* and *A. carbonarius*. Sixty out of the 231 yeast strains showed an effect of slowing down or inhibiting growth of the three tested molds. Thirty-six out of 60 selected antagonistic yeasts were isolated from *V. vinifera* ssp. sylvestris, 9 from the biodynamic vineyard, 1 from the organic vineyard, and 4 from the conventional one (**Table 2**). The majority of the strains with antagonistic activity were isolated from wildlife vines (53%), followed by those isolated from the biodynamic (14.5%), the organic farming system (6.2%), and the conventional (4.7%) vines (**Table 2**).

After the preliminary assay, a second *in vitro* test was performed. It consisted of a test on solid medium where Petri-dishes were plated with a yeast cell-top agar suspension and the mold spores were spotted on the center of the plate. The percentage of the mycelium growth was calculated for each yeast strain against each mold (Table S1, Supplementary Material 1). Twenty yeast strains (plus the control) out of 60, which passed the first screening, inhibited the 100% of hyphal growth of the three tested molds in comparison with the control. Among these, 18 strains were isolated from the wild vines and belonged to *H. uvarum* (9), *M. guilliermondii* (2), *P. kluyveri* (2), *S. cerevisiae*, *H. clermontiae*, *M. fructicola*-like yeast strain, *M. viticola,* and *C. californica* species, and two strains were isolated from the biodynamic vines and were ascribed to *A. pullulans* and *V. carnescens* species (**Table 2**). These 20 yeast strains were selected for the successive tests in order to understand the nature of antagonistic activities.

TABLE 1 | Yeast species occurrence and distribution of the isolated and identified from *V. vinifera* ssp sylvestris and from the different vine cultivars of *V. vinifera* ssp vinifera (conventional, biodynamic, and organic), GenBank accession numbers of the deposited sequences and The Centraalbureau voor Schimmelcultures (CBS) and D1/D1 Genbank accession numbers of the most similar types.

Isolate			Most similar type/reference strain		Source			
Strain code	**D1/D2 accession no.**	**ITS accession no.**	**Taxonomic name**	**D1/D2 accession number**	**Conventional vineyard**	**Biodynamic Vineyard**	**Organic Vineyard**	***Vitis vinifera* ssp. sylvestris**
FZ02	MF926292	MF783894	*Aureobasidium pullulans* CBS584.75	KT361587.1	46	15	9	1
CABMC2A	MF927682	MF770161	*Candida californica* CBS989	KY816896	–	–	–	1
FZ03a	MF783064	–	*Filobasidium stepposum* CBS10265	KY107724.1	2	–	–	–
HB09c	MF783066	–	*Filobasidium wieringae* CBS1937	KY107733	–	–	–	1
CABMB1A	MF783060	–	*Hanseniaspora clermontiae* CBS8821	EU272040	–	–	–	1
HURM6B	MF926297.1	–	*Hanseniaspora* ssp CBS276	KY107853	–	–	–	4
CAMB9A	MF783054	–	*Hanseniaspora uvarum* CBS9790	KJ794689	17	34	1	28
NUR3AM	MF926296	–	*Hyphopichia pseudoburtoni* CBS2455	KU609072	–	–	–	1
ROMA10*	MF783057	–	*Metschnikowia fructicola* CBS8853	AF360542	–	–	–	5
CABM7C*	MF783068	–	*Metschnikowia pulcherrima* CBS5833	JN083816	9	8	1	1
CABM9C*	MF783069	–	*Metschnikowia* spp CBS5536	KM350710	–	–	–	5
ROMAM1A*	MF783062	–	*Metschnikowia viticola* CBS9950	KC859919	–	–	–	2
SEHMA2	MF783056	–	*Meyerozyma caribbica* CBS2829	KX507035	–	–	–	1
SEHIB8	MF783055	–	*Meyerozyma guilliermondii* CBS8105	KY108543	–	–	–	4
HB01a	MF926291	MF783893	*Papiliotrema flavescens* CBS942	AB035042	4	–	1	–
CABM8C	MF926294	MF783895	*Pichia fermentans* CBS5663	EF550234	–	–	–	1
SEMA6B	MF783059	–	*Pichia kluyveri* CBS7274	KY108823	–	–	–	4
SEHM2A	MF927685	MF783892	*Rhodosporidium babjevae* CBS322	AF387771	–	–	–	1
EP02c	MF783058	MF927679	*Rhodotorula glutinis* CBS2889	KY109044	3	4	1	–
HURM4A	MF783067	MF927680	*Rhodotorula mucilaginosa* CBS482	KY109140	–	–	–	1
SEHUM7B	MF783065	MF784281	*Rhodotorula nothofagi* CBS9091	AF444736	–	–	–	1
ARIM1B	MF926295	MF783896	*Rhodotorula paludigena* CBS4477	KY109146.1	–	–	–	1
CABMA3A	MF783053	–	*Saccharomyces cerevisiae* CBS2963	KF214442	–	–	–	1
SEHM1C	MF770267	–	*Scheffersomyces stipitis* CBS7126	KY109584.1	–	–	–	1
PIEM5B	MF783061	–	*Schwanniomyces polymorphus* CBS6456	KY109627	–	–	–	1
HB02b	MF926293	MF783891	*Vishniacozyma carnescens* CBS973	AB035054	4	1	3	–
			Total:		85	62	16	67

** This table shows the most probable yeast strain according to the compared databased belonging to the Metschnikowia clade, but these yeast strains cannot be assigned unequivocally to one of the species in the clade.*

TABLE 2 | *In vitro* dual assays of yeast strains against mycelial growth of *B. cinerea*, *P. expansum,* and *A. carbonarius*.

Source	Isolates from grapes	Isolates with inhibitory capacity at preliminary *vitro* assaying	% of isolates with inhibitory capacity at preliminary *vitro* assaying	Isolates with inhibitory capacity at second *vitro* test	% of isolates with inhibitory capacity at second *vitro* test	% of isolates with inhibitory capacity
Wildlife vines	67	42	62.7	18	42.9	26.9
Biodynamic vineyard	62	11	17.7	2	18.2	3.2
Organic vineyard	16	1	6.2	0	0	0
Conventional vineyard	85	6	7.1	0	0	0
Total isolates	230	60	26.1	20	33.3	8.7

In the first in Vitro assaying, all isolates are present. At second in Vitro test only the positive at first are shown.

Evaluation of the Minimum Inhibiting Concentration (MIC)

MICs were determined in triplicate for all yeast strains selected after dual assays against the different molds. The evaluation of the MIC revealed that the 20 yeasts significantly reduced the progress of hyphal growth of *B. cinerea* and *P. expansum* at a concentration of 10^5 cells/mL, and 10 (5 *H. uvarum*, 1 *P. kluyveri*, 1 *M. guilliermondii*, 1 *H. clermontiae,* and 1 *S. cerevisiae*) at a concentration of 10^3 cells/mL both under the mentioned growth conditions (**Table 4**). However, the occurrence of *A. carbonarius* was completely reduced by only 14 yeast strains at a concentration of 10^6 cells/mL. Only two yeast strains (1 *H. uvarum* and 1 *S. cerevisiae*) were able to protect grapes or to compete for the nutrients against *A. carbonarius* at a concentration of 10^3 cells/mL and under the same growth conditions of *B. cinerea* and *P. expansum* (**Table 4**). The yeasts that were able to protect grapes or to exhaust the medium from all the assayed molds were those isolated from *V. vinifera* ssp. sylvestris.

Killer Character Assay

From over the 20 yeast strains assayed for the killer character, only *S. cerevisiae* displayed a slightly killer phenotype (**Table 3**).

Enzymatic Tests

All yeasts that passed the dual test were evaluated for extracellular enzymatic activities (β-1, 3-glucanase, proteolytic, and pectinolytic activities). Twelve out of the 20 yeast strains were able to hydrolyze at least one of the assayed compound (milk proteins, pectin, glucan, and chitin). Only five yeast strains (4 *M. fructicola*-like yeast strains and 1 *P. kluyveri*) showed all the enzymatic activities (**Table 3**).

Production of Volatile Organic Compounds (VOCs) and Hydrogen Sulfide Release

Percentage data concerning production of VOCs and hydrogen sulfide release among the 20 yeast strains selected showed that 10 yeast strains (3 *H. uvarum*, 4 *M. fructicola*-like yeast strains, 2 *M. guilliermondii,* and 1 *S. cerevisiae*) evidenced the highest values of growth inhibition. These values significantly differed ($p < 0.05$) from the control and the other yeast strains analyzed (**Table 3**).

Biofilm Formation

Only yeast strains of *H. uvarum* (1), *P. kluyveri* (1), *V. carnescens,* and *A. pullulans* proved to be able to form biofilm by the adhesion to polystyrene 96-well plate surface (O.D. > 0.1) after 3, 48, and 72 h of incubation (**Table 3**).

Effect of Iron Concentration on the Inhibitory Activity of the Yeast Strains

Antagonistic activity of most of the selected strains were not significantly influenced by tested $FeCl_3$ concentrations showing that inhibition activity of these yeasts against *B. cinerea* and *A. carbonarius* were not related with iron competition (**Table 3**). On the other hand, the activity of the *P. kluyveri* strains resulted iron-sensitive at a concentration of 20 μg/mL of $FeCl_3$. The potential yeast strain ROMA10 (*presumably M. fructicola*) always produced red pigments in absence or presence of $FeCl_3$ at different concentrations on PDA plates without affecting the pigment coloration or the inhibition of the mold. Regarding the species *A. pullulans,* depending on the concentration of iron, yeast colonies, and haloes pigmentation turned from pale white to maroon, but in absence of $FeCl_3$ colonies were not pigmented and the halo was not visible. These findings will be argued in the discussion section.

Effect of Other Metabolites Released by Yeast Strains on Mold Growth

Yeast primary or secondary metabolism generates numerous compounds as products of the transformation of the carbon, nitrogen, or sulfur sources. Two of the most common substances released are acetic acid and hydrogen sulfide that have antimicrobial effect. **Table 3** shows that *M. fructicola*-like strain, *H. uvarum* (2 strains), *M. guilliermondii* (1 strain), *S. cerevisiae,* and *C. californica* species are able to produce these compounds probably affecting the mold development.

In Vivo Assays for Inhibitory Activity

Efficacy of Yeast Strains in Controlling Mold Infection on Grape Berries

The results of the efficacy of the 20 selected strains in reducing molds berry rots are reported in **Table 3**. *P. kluyveri* (2 strains), *H. uvarum* (2 strains), *H. clermontiae* (1 strain), and *M. guilliermondii* (1 strain) revealed the highest efficacy in

TABLE 3 | Phenotypical assaying for yeast antagonistic activity against molds and their volatile organic compounds (VOCs) referred to mycelial growth reduction of *B. cinerea*, *P. expansum*, and *A. carbonarius*.

Species	Strain	D1/D2 Accession no.	VOCs[a] (%)	Protease	Pectinase	Glucanase	Chitinase	Glucosidase	Killer activity	Acetic acid production[b]	H_2S released	Iron depletion[c]	Biofilm formation[d]
A. pullulans	FZ02a	MF926292	28.0	−	+	+	+	−	−	0.3	+	Positive with *Botrytis*	0.110
C. californica	CABMC2A	MF927682	45.0	−	−	−	−	−	−	0	+	Positive with *Botrytis*	0.030
H. uvarum	SEHMA6A	MF783054	31.0	−	+	−	−	−	−	0	−	Positive with *Botrytis*	0.042
H. uvarum	CABM8A	MF926284	44.5	−	+	−	−	−	−	0.1	+	Positive with *Botrytis* and *Aspergillus*	0.010
H. uvarum	CABCM1A	MF926285	35.8	−	+	−	−	−	−	0.2	−	Positive with *Botrytis*	0.100
H. uvarum	CAMM3A	MF926286	34.8	+	+	−	−	−	−	0.1	−	Positive with *Botrytis*	0
H. uvarum	CAMM6A	MF926287	40.5	−	−	−	−	−	−	0.3	−	Negative	0.010
H. uvarum	SEHI3C	MF927683	25.8	−	−	−	−	−	−	0.1	−	Positeive with *Botrytis*	0.030
H. uvarum	SEHI1C	MF926288	21.0	−	+	−	−	−	−	0	+	Positive with *Botrytis*	0.080
H. uvarum	SEHM7C	MF926289	26.3	−	−	−	−	−	−	0.1	−	Positive with *Botrytis* and *Aspergillus*	0.150
H. uvarum	CAMB9A	MF926290	27.7	−	−	−	−	−	−	0	−	Negative	0.034
H. clermontiae	CABMB1A	MF783060	18.7	+	−	−	−	−	−	0	−	Positive with *Botrytis*	0.011
H. uvarum	Control	MF801365	28.7	+	−	−	−	−	−	0.3	+	Negative	0.033
*M. fructicola**	ROMA10	MF783057	28.3	+	−	−	−	−	−	0	−	Positive with *Botrytis*	0.070
M. guilliermondii	CABM1A	MF927684	44.5	−	+	−	−	−	−	0.2	+	Negative	0.010
M. guilliermondii	SEHIB8	MF783055	37.0	+	+	−	−	−	−	0.2	+	Positive with *Botrytis*	0.027
*M. viticola**	ROMMA1A	MF783062	46.5	+	−	−	−	−	−	0	−	Positive with *Botrytis*	0.050
P. kluyveri	SEHMA6B	MF783059	26.7	−	−	−	−	−	−	0	+	Positive with *Botrytis* and *Aspergillus*	0.014
P. kluyveri	CABMC6C	MF926283	29.5	+	−	−	−	−	−	0	−	Positive with *Botrytis*	0.360
S. cerevisiae	CABMA3A	MF783053	40.0	−	−	−	−	+	+	0.1	+	Positive with *Botrytis*	0.010
V. carnescens	HB02b	MF926293	28.0	−	−	−	−	−	−	0	−	Positive with *Botrytis*	0.110

**This table shows the most probable yeast strain according to the compared databased belonging to the Metschnikowia clade, but these yeast strains cannot be assigned unequivocally to one of the species in the clade.*

[a]*The percentage is calculated: (M – Mwy)/M*100 where M is the mold growth (cm) without antagonistic yeast on the plate and Mwy is the mold growth in presence of the antagonistic yeast on septet plates (cm). The percentage represents the reduction of mold grown caused by yeast VOCs.*

[b]*Values are expressed in centimeters (diameter of the halo of the positive acetic acid-producing yeast strains on the plate) a strain of Acetobacter was used as positive control.*

[c]*Positive is when in presence of iron the yeast decreases its antagonistic activity; Negative is when the antagonistic activity of the yeast is the same in presence or in absence of iron.*

[d]*The values are expressed as the average of the absorbance at 590 nm of three well-subtracted for the control test values.*

TABLE 4 | Disease incidence by *A. carbonarius*, *B. cinerea,* and *P. expansum* after simultaneous inoculation with different concentrations of yeast strains on PDA-agar after 5 days at 25°C under constant light.

Species	Strains	*A. carbonarius*				*B. cinerea*				*P. expansum*			
		10^{6*}	10^5	10^4	10^3	10^6	10^5	10^4	10^3	10^6	10^5	10^4	10^3
A. pullulans	FZ02a	−	−	−	−	+	−	−	−	+	+	+	+
C. californica	CABMC2A	−	−	−	−	+	−	−	−	+	+	+	+
H. clermontiae	CABMB1A	+	−	−	−	+	+	+	+	+	+	+	+
H. uvarum	SEHMA6A	+	−	−	−	+	+	+	+	+	−	−	−
H. uvarum	CABM8A	+	−	−	−	+	+	+	+	+	+	−	−
H. uvarum	CABCM1A	+	+	−	−	+	+	+	+	+	+	+	+
H. uvarum	CAMM3A	+	+	−	−	+	+	+	+	+	+	+	+
H. uvarum	CAMM6A	+	−	−	−	+	+	−	−	+	+	+	+
H. uvarum	SEHI1C	+	−	−	−	+	−	−	−	+	+	+	+
H. uvarum	SEHM7C	+	−	−	−	+	+	−	−	+	+	+	−
H. uvarum	CAMB9A	+	+	+	+	+	+	+	+	+	+	+	+
H. uvarum	SEHIC3	−	−	−	−	+	+	+	+	+	+	+	+
H. uvarum	Control	−	−	−	−	−	−	−	−	−	−	−	−
M. guilliermondii	CABM1A	+	+	−	−	+	+	+	+	+	+	+	−
M. guilliermondii	SEHIB8	+	−	−	−	+	+	+	+	+	+	+	+
P. kluyveri	SEHMA6B	+	−	−	−	+	+	+	−	+	+	+	+
P. kluyveri	CABMC6C	+	+	−	−	+	+	+	+	+	+	+	+
S. cerevisiae	CABMA3A	+	+	+	+	+	+	+	+	+	+	+	+
V. carnescens	HB02b	−	−	−	−	+	−	−	−	+	+	+	+

*Values are expressed as (+) if yeast strains were able to inhibit the total growth of the mold over a particular concentration and (–) if yeast strains were not able to inhibit mold growth. Values were obtained from three trials.*The values are expressed in CFU/mL.*

TABLE 5 | Comparative *in vivo* test of the most suitable yeast strains against molds vs. a commercial chemical fungicide.

Species	Strains	*A. carbonarius*			*B. cinerea*			*P. expansum*			Mean
H. uvarum	SEHMA6A	3	3	3	3	3	3	3	3	3	3.00
H. uvarum	CABMB9A	2	3	3	3	3	3	3	3	3	2.89
P. kluyveri	SEHMA6B	2	2	2	2	1	1	1	3	2	1.78
Commercial fungicide		1	2	2	3	3	3	2	2	3	2.33
Control		4	4	4	4	4	4	3	3	3	3.67

The disease severity was evaluated by a visual score "1-to-4" (1: no visible symptoms; 2: soft rot; 3: formation of mycelium; 4: sporulation of mold) according to Parafati et al. (2015).

reducing mold infection and growth caused by *B. cinerea*, *A. carbonarius,* and *P. expansum*. On the contrary, a strain of *M. guilliermondii* showed the worst result in controlling molds decay on grape-berries.

Comparison of the Inhibitory Effect with Chemical Pesticide by *In Vivo* Test

The three yeast strains which showed a better antagonistic effectiveness against the studied molds taking into account the above described experiments, were subjected to a comparative *in vivo* test with a commercial chemical fungicide used against *B. cinerea* and other molds including *P. expansum* and *A. carbonarius* (**Table 5**). In this case, the strain *P. kluyveri* SEHMA6B proved to be more effective than the chemical fungicide used under the proposed growth conditions.

DISCUSSION

The control of fungal diseases and mycotoxins contamination during grape maturation and post-harvesting is currently based on treatments with chemical fungicides. However, the environmental dispersion, the progressive loss of effectiveness, the emergence of resistant strains, and the increasing level of residues in table grape and wine (Marssat et al., 2016), have led the European Union to restrict the use of these compounds, addressing the researchers toward innovative and eco-friendly protocols to face the problem. In agreement with the recommendations pursued by UE Directive 128/2009, this work has been focused on the exploration of the natural antagonistic potential of 241 yeasts isolated from grape samples of *V. vinifera* ssp. sylvestris and *V. vinifera* ssp. vinifera against *B. cinerea*, *A. carbonarius,* and *P. expansum*. These molds are spoilage agents of the berries, both in vineyard after the veraison and

during the over-ripening practices, by rotting the grape bunches that cause the falling of the fruit quality and, in the case of *Aspergillus* and *Penicillium* genera, a threat to food safety due to the release of mycotoxins. According to Wilson and Wisniewski (1989), biocontrol is the application of selected microorganisms with antagonistic activity against other ones and their usage at large-scale to reduce the impact of chemical synthesis pesticides on human health and environment. Many papers report the discovering of novel microbial strains with antifungal properties, proposing them as biocontrol strains against certain molds (Marssat et al., 2016). Although some natural fungicides have been marketed, they can fail in field practices since climatic conditions affect the establishment, survival and activity of the biocontrol agents (Benbow and Sugar, 1999). Yeasts are structurally and functionally heterogeneous because of their differential expression of genes, in a way that epigenetic factors, such as the host environment or abiotic external factors influence the down/up regulation of the gene expression, changing the behavior of yeast populations and their interactions (Spadaro and Droby, 2016). The present investigation shows that yeast strains isolated from various environments have significant differences on the effectiveness against three potentially harmful fungi. To our knowledge, this is the first report in which yeasts isolated from *V. vinifera* ssp. sylvestris and from biodynamic or organic grapevines have been assessed for potential antagonist ability against *A. carbonarius*, *B. cinerea,* and *P. expansum*.

Our results pointed out that there is a greater number of species found on wildlife vines (23), compared to cultivated ones, with only seven species. This is in line with other studies, which demonstrated that the biodiversity level of yeasts community is influenced by human activities (Cordero-Bueso et al., 2011a,b, 2014, 2017; Martins et al., 2014; Drumonde-Neves et al., 2016). In addition, *S. cerevisae* was also isolated on wildlife grape surfaces. Previous studies on yeast diversity from cultivars or overwintering vines show that *Saccharomyces* genus is either absent on grapes or found in a small number and incidence (Mortimer and Polsinelli, 1999; Torija et al., 2001; Sipiczki, 2016). The results obtained from the preliminary *in vitro* dual assay have clearly disclosed how most isolates collected from wildlife vines (18 strains) are able to inhibit the mold growth vs. the isolates from managed cultivars (only two strains in biodynamic farming). Interestingly, yeast strains, which passed the preliminary tests, have been isolated in two ecosystems where the microbial antagonism against molds could only be produced by the associate microbiota onto grape-berries or natural barriers of the plant that hinder the entry of fungal pathogens. Consequently, *H. uvarum*, *H. clermontiae*, *M. guilliermondii,* and *Pichia kluyveri* strains, all of them isolated from *V. vinifera* ssp. sylvestris, could play a pivotal role as biocontrol agents in the natural environment. These data cannot be compared with the current literature since this is the first time that isolates from wildlife vines are studied with this aim. It is possible to hypothesize that the observed differences in microbiota structure between grapes from wildlife vines and cultivated ones can be due to the use of synthetic or natural pesticides in vineyards or the isolation from overwintering vineyards, resulting in a diverse selective pressure on resident microorganisms (Sipiczki, 2006, 2016; Cordero-Bueso et al., 2011a, 2014; Brysch-Herzberg and Siedel, 2015). The higher yeast biodiversity found in samples from native conditions, highlighted in this work, might have been because the natural environment is hostile for the mold development. Moreover, it seems reasonable to think that molds exposed to repetitive doses of synthetic fungicides can acquire, modify, or adjust genetic characters that provide them an increase in the resistance.

The minimum inhibitory concentrations (MICs) assays, defined as the lowest concentrations of yeasts resulting in complete growth inhibition of the molds, have shown that a concentration of 10^5 cells/mL is enough to reduce the progress of *B. cinerea* and *P. expansum* by all yeast strains. The mold *A. carbonarius* needed a concentration of 10^6 cells/mL to be inhibited. These concentrations are considerably lower than those found for other antagonistic yeasts (Chanchaichaovivat et al., 2007; Zhang et al., 2007; Nally et al., 2012). However, further experiments are required to evaluate the influence of the growth condition on the MIC values on field.

Since several mechanisms of action are involved in the biocontrol activity of the antagonistic yeasts, we have examined the main modes of actions, such as iron depletion, cell wall degrading enzymes, diffusible, and volatile antimicrobial compounds, and biofilm formation on the 20 selected yeast strains. Within this group *M. guilliermondii*, *H. clermontiae*, *P. kluyveri*, *H. uvarum*, *A. pullulans,* and the yeast strain ROMA10 (*M. fructicola*-like strain*)* strains proved to release lytic enzymes potentially capable of hydrolyzing the fungal cell wall. Among these species, it is well-known that *A. pullulans* is able to produce β-1,3 glucanase, and chitinase active on *Monilinia laxa*, *B. cinerea,* and *P. expansum*, especially when the mold wall represents the sole carbon source (Zhang et al., 2009).

The yeast metabolism leads to the formation of acetate and ethyl acetate, which are by-products with inhibitory action against molds in storing cereals (Fredlund et al., 2004). Furthermore, some yeasts can emit volatile compounds that inhibit the development of molds, as described by Parafati et al. (2015) where the growth of *B. cinerea* was counteracted by *S. cerevisiae*. In our experimental conditions, the species *H. uvarum*, *S. cerevisae,* and *M. guilliermondii* were able to release sufficient levels of acetic acid and hydrogen sulfide (evaluated qualitatively) to cause inhibition to mold growth. Likewise, some *M. fructicola-like* strains were capable of preventing the development of molds through the emission of volatile compounds. Regarding this species there are no examples in the literature, despite the report of a commercialized product used as biocontrol agent (Shemer, Bayer CropScience, AG, Germany).

Little is known about the role of biofilms in the biocontrol activity of yeast used to control fungal diseases and the mechanisms involved in their formation. In this work, *H. uvarum*, *P. kluyveri*, *V. carnescens,* and *A. pullulans* strains revealed the capability to form biofilm. Previous studies carried on the species *S. cerevisiae* showed that the ability to adhere to a surface was related to the production of extracellular polysaccharides and molecules belonging to glycoproteins family implicated in this action and in the grape wounds protection (Reynolds and Fink, 2001; Parafati et al., 2015). Yeasts cells with

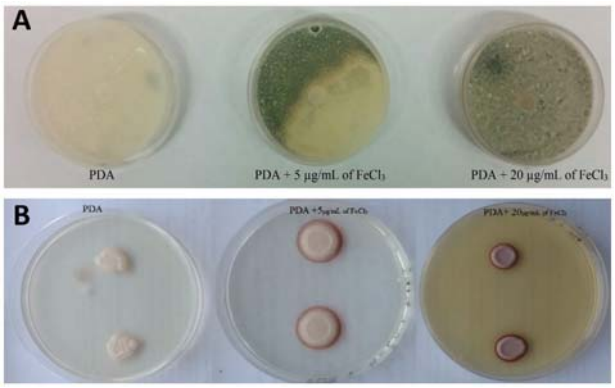

FIGURE 2 | (A) Biocontrol activity of *P. kluyveri* SEHMB8A against *P. expansum* in PDA at different concentration of iron. The activity of this yeast strain is iron-sensitive in presence of an excess of iron the strain loses its antagonism. **(B)** Depending on the concentration of iron added to the PDA medium, the species *A. pullulans* turned from pale white to maroon, but in absence of $FeCl_3$ the halo was not visible.

the ability to form biofilm are recognized as most effective in limiting pathogen growth being able to colonize more efficiently the inner of grape wounds (Ianiri et al., 2013).

Iron is essential for fungal growth and pathogenesis, thus, competition for this metal is functional for counteracting of pathogenic molds. Sipiczki (2006) and Spadaro and Droby (2016) reported this action on strains belonging to the genus *Metschnikowia* that were capable of stopping mold development in crop areas through an iron deficiency mechanism. In the tests we carried out, the presence of iron in growth medium modified the inhibitory properties of the antagonist yeasts (**Figure 2A**). In particular, for *B. cinerea*, when an excess of iron was present the mold was able to develop contrary to what was happening in growth media without $FeCl_3$, where the action of yeast prevented its development. Spadaro and Droby (2016) affirmed that some *M. fructicola* strains were able to produce the red pigment pulcherrimin surrounding its colonies in presence of $FeCl_3$ in the growth medium. However, in accordance to Sipiczki (2006), Sipiczki et al. (2013), Brysch-Herzberg and Siedel (2015), Lachance (2016), and Sipiczki (2016) these yeast strains could not be suitable for the delimitation of the species *M. fructicola*. This species is not distinguishable from *M. andauensis* and other species of the *M. pulcherrima* clade because of a possible heterogeneity of the rRNA repeats. Thus, we will consider that these yeast strains are inside of the *M. pulcherrima* clade but not as confirmed *M. fructicola* species. Previous studies investigating the mechanism of antifungal antagonism of pulcherrimin-producing *Metschnikowia* strains claimed that iron immobilization by pulcherrimin (and thus antifungal activity) was suppressed by iron depletion (Sipiczki, 2006). However, in our study, yeast strain ROMA10 (presumably identified as *M. fructicola*) was able to produce pulcherrimin-like substances in presence of $FeCl_3$ at the studied concentrations. This result was also previously observed on apple fruits (Saravanakumar et al., 2008). Interestingly, our yeast strain FZ02 identified as *A. pullulans*, did not show halo without the $FeCl_3$ addition on the medium, but colonies showed a pink halo at low iron concentration and then they turned to red-maroon at high iron concentrations (**Figure 2B**). This observation is in accordance with Chi et al. (2013) that reported that in a medium supplemented with iron, the colonies of *A. pullulans* turned to brown. They supposed that the iron was chelated by the secreted siderophores and considerable amount of the intracellular siderophores was responsible for brown colonies. However, further studies are necessary to elucidate both findings described above. The antagonistic potential of the 20 yeast strains selected after *in vitro* tests was further proven on wounded grape berries inoculated with *A. carbonarius*, *B. cinerea,* and *P. expansum*, *P. kluyveri*, *H. uvarum*, *H. clermontiae,* and *M. guilliermondii* strains exhibited the best efficacy in reducing the development of tested mold diseases. As reported by Parafati et al. (2015), *S. cerevisiae* species reveals to be less efficient than the non-*Saccharomyces* to hamper the fungal growth, probably due to its difficulty to multiply on grape wounds. Nevertheless, these results display that the cumulative effects of different antagonistic activities detected by the *in vitro* tests are not sufficient to explain the outcome of the most performant strains on grape berries (*in vivo* experiments). The efficacy of the yeast strains which showed the greatest *in vivo* action on grape berries, were also compared with a fungicide formulation (37.5% Cyprodinil and 25% Fludioxonil) normally used against *Botrytis* and as secondary rots *Aspergillus* spp. and *Penicillium* spp., according to the supplier's recommendations. We decided to exclude those isolates that show the VOCs production and that release extracellular enzymes, taking into account that the emission of certain compounds, and hydrolytic enzymes by yeasts could alter the balance of the resident microbiota and destabilize the microbial composition of the must. Surprisingly, *P. kluyveri* strain SEHMA6B was more effective than the commercial fungicide, particularly against *Botrytis* (**Figure 3**). Considering that gray mold decay is the main problem of pre-harvesting, the application of this yeast strain in the field could be even more interesting. Moreover, in a recent study (Sipiczki, 2016) a grape-born *P. kluyveri* strain was tested against *Botrytis* and *S. cerevisiae*. It was active against *Botrytis* but no detectable inhibitory effect on *Saccharomyces*. Other studies have demonstrated that this species is unable to compete with *S. cerevisiae* during fermentation (Cocolin and Ciani, 2014), thus, *P. kluyveri* could be used as biocontrol without alter the fermentation processes. Interestingly, the *P. kluyveri* strain tested by Sipiczki (2016) was isolated from mummified grapes which indicates that it prefers harsh conditions. This fact makes us hypothesize that *P. kluyveri* would be able to cope in the different conditions in field. Nevertheless, further studies are needed to test the antagonistic activity of *P. kluyveri* in field to verify if in the conditions that occur in the vineyard such as temperature swings, high humidity, water, solar radiation, and interaction with the resident microbiota it is able to be effective in counteracting the growth of molds.

Actually, several yeast strains tested in the *in vitro* trials, when air exchange was limited, proved to be effective against molds, while under the *in vivo* outdoor conditions turned out to be ineffective. The main studies on volatile substances are aimed at storing, packaging, and transporting fruit and vegetables

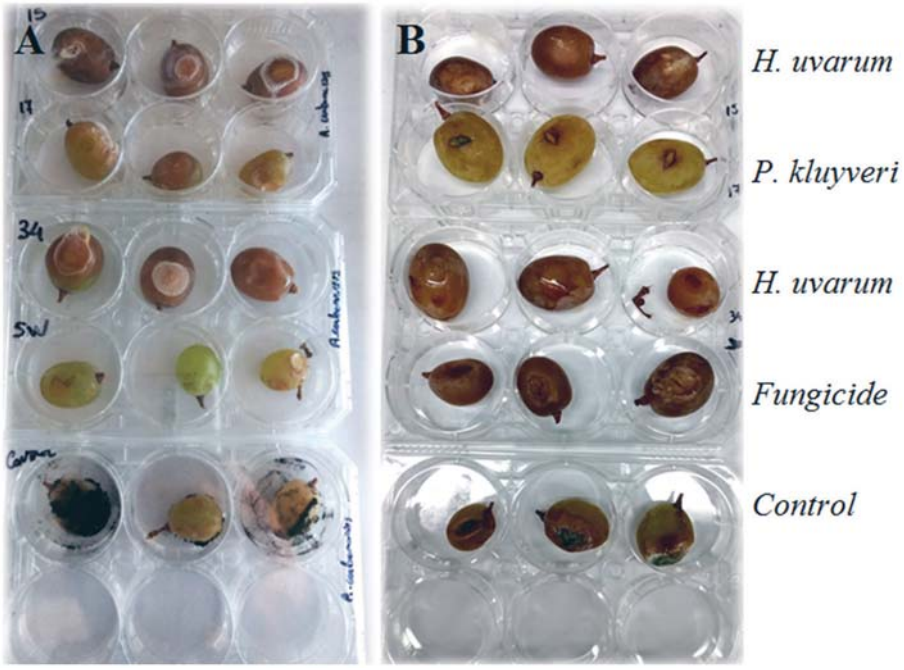

FIGURE 3 | Comparison of the three selected antagonistic yeast strains against *A. carbonarius* **(A)** and *B. cinerea* **(B)** and the commercial fungicide. Line 1: Grapes soaked with *H. uvarum* strain 1, Line 2: Grapes soaked with *P. kluyveri* SEHMB8A, Line 3: Grapes soaked with *H. uvarum* SEHMA61 strain 2, Line 4: Grape soaked with commercial fungicide, Line 5: Grapes without treatment.

(Gomes et al., 2015). From a commercial point of view, it is important to understand the ways in which yeast acts to develop an appropriate formulation and method of application (Spadaro and Droby, 2016). The ability to compete with some nutrient yeast, for example for iron or biofilm formation, is the desired interaction. For these reasons, two isolates of *H. uvarum* and one of *P. kluyveri*, which do not produce hydrolytic enzymes, have been used for the final test with the phytopoietic drug.

Though variable performances in field can be a significant constraint for its practical implementation (Stewart, 2001; Elmer and Reglinski, 2006), the interest in the use of bio-control is renewed because of the recent normative (Directive 2009/128/EC), by matching the specific requirements of International Organization of Vine and Wine for the sustainable production of wine.

In conclusion, this investigation on antagonism patterns in new yeast isolates, over all from *V. vinifera* ssp. sylvestris, can constitute a promising source of knowledge and experience to set strategies in preventing or reducing harvested commodity damages and to test the use of selected yeast strains as a substitutive of the chemical fungicide.

AUTHOR CONTRIBUTIONS

GC contributed to the design of the work, to the yeast isolation, and identification, to the *in vitro* assays for antagonistic activity, to the analysis and to the interpretation of data for the work, to draft the work and revising it, NM contributed to the *in vitro* assays for antagonistic activity, to *in vivo* assays for inhibitory activity, to draft the work, and revising it, DM to the samples collection for yeast isolation, RF and JC contributed to draft the work and revising it, FV contributed to the yeast identification, IV contributed to the design of the work, to the interpretation of data for the work, to draft the work, and revising it for important intellectual content, and ensured that that questions related to the accuracy or integrity of any part of the work were appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2017.02025/full#supplementary-material

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Chapter 10

Ampelography

Ampelographic Description of Wild Grape Accessions

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Introduction

"Ampelography" is a science (and art) for studying grapevine varieties and species for their description, identification and classification based on objective methods and aesthetic ability. It is a tool in the hand of the researchers to describe biodiversity of any target trait linked to a specific gene pool at different taxonomical level and to see how this gene pool is similar or distinct from another ones. Thanks to this methodology it became possible to make acknowledge and classifications of wide diversity of genus *Vitis* L.

The term "Ampelography" was born in the 17th century to describe grape varieties and very soon it became a leader part of viticultural discipline. The 19th century was the most significant period for development of this discipline – invasion of American pathogens to Europe increase need for description of grape varieties under the risk of extinction in the second half of that century. Since that time the botanical method for description of grape organs demonstrated significant progress of study grape diversity. In parallel, visual ways of description by drawing and after by photography were developed, enriching informative value for publication of ampelographic books (*for example*: Viala & Vermorel, 1910, Ampelography, 1946-1970, Del Zan et al. 2009, Schneider et al. 2012). Recent achievement of "Molecular ampelography" (IFV, 2022) increased resolution of ampelography using additional methods of investigation.

Description of wild grapevine accessions often is done on the samples collected after *in situ* expedition and originated from various ecological conditions (Benito et al. 2017). In spite of high importance of similar materials for basic morphological description, the detail study of genotypes in more accurate manner can be done in a field collection being under the similar climatic, soil and vineyard management conditions (Maghradze et al. 2021, Benito et al. 2017, Derosas et al. 2010).

The aim of the research was studying of the wild grape accessions of Georgian origin in the Jighaura field collection by using methods of ampelography, biochemistry, biology and agronomy with specific tasks: i) Ampelographic description and agronomical study of wild grape accessions; ii) Eno-carpological characterization of grapes and musts; iii) Analysis of grape anthocyanins and polyphenols; iv) Monitoring of phenological phases of grapevine development; v) Making photo records of grapevine organs.

Materials and Methods

Plant materials. It was included 41 accessions of wild grapevine *Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi in this study. These accessions represent 20 populations from Eastern and Western parts of Georgia. The accessions were discovered in Georgia during expeditions of 2003-2013 (Figure 1).

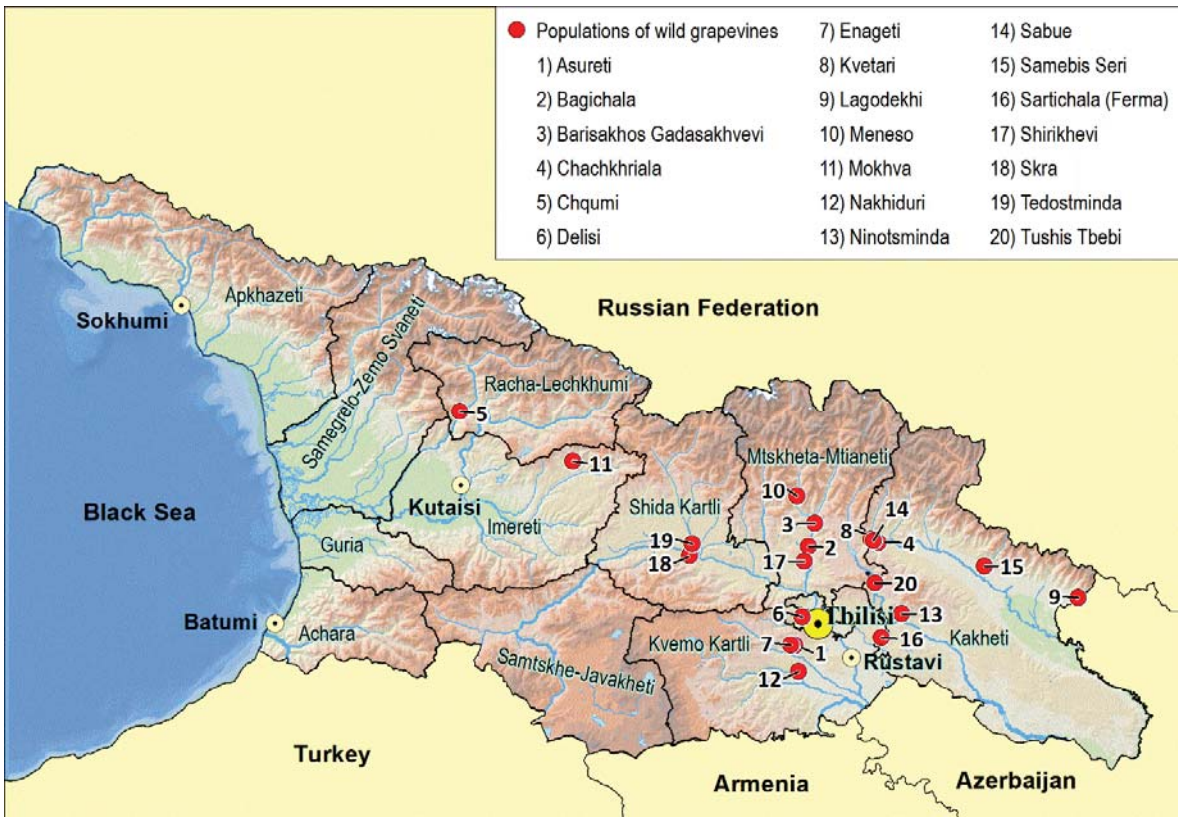


Figure 1. Location of the discovered population in Georgia (*the maps has been prepared by N. Kvaliashvili*)

Based on discovered plants it was established the first set of grafted field collection in Jighaura (village Saguramo, Mtskheta district) in 2014. The genotypes included in this study were selected from this collection (Table 1). Each accession in the collection is represented with 1-6 plants. The data presented here were collected in the period of 2019-2021.

Table 1. Origin and the number of studded accessions

#	Population name	Origin			Number of studded accessions
		Part of Georgia	Viticultural region	District	
1	Asureti	Eastern	Kartli, Lower	Tetritskaro	1
2	Bagichala	Eastern	Kartli, Inner	Dusheti	3
3	Barisakhos Gadasakhvevi	Eastern	Kartli, Inner	Dusheti	1
4	Chachkhriala	Eastern	Kakheti	Akhmeta	1
5	Chqumi	Western	Lechkgumi	Tsageri	4
6	Delisi	Eastern	Kartli, Inner	Tbilisi	1
7	Enageti	Eastern	Kartli, Lower	Tetritskaro	1
8	Kvetari	Eastern	Kakheti	Akhmeta	2
9	Lagodekhi	Eastern	Kakheti	Lagodekhi	1
10	Meneso	Eastern	Kartli, Inner	Dusheti	1
11	Mokhva	Western	Imereti	Sachkhere	1
12	Nakhiduri	Eastern	Kartli, Lower	Bolnisi	1
13	Ninotsminda	Eastern	Kakheti	Sagarejo	4
14	Sabue	Eastern	Kakheti	Akhmeta	2
15	Samebis Seri	Eastern	Kakheti	Kvareli	1
16	Sartichala (Ferma)	Eastern	Kakheti	Sartichala	3
17	Shirikhevi	Eastern	Kartli, Inner	Dusheti	2
18	Skra	Eastern	Kartli, Inner	Gori	1
19	Tedostminda	Eastern	Kartli, Inner	Gori	6
20	Tushis Tbebi	Eastern	Kakheti	Sagarejo	1

The vineyard. The description of accessions provided in this book was done in the grape germplasm repository located in the village Jighaura of Mtskheta district. The Jighaura collection (VIVC code: GEO038) is sited in Shida (Inner) Kartli province of Georgia (Latitude 41.55, Longitude 44.46, Elevation 585 m a.s.l.). It belongs to the Scientific-Research Center of Agriculture, was established in 2008, named after Acad. Solomon Cholokashvili and preserves more than 1000 accessions of *Vitis*. Establishment of the wild grape collection in Jighaura started in 2014 with the goal to accumulate discovered in Georgia wildly growing grapevines for preservation and following research.

The vineyard is planted in a plain area, with a typical alluvial carbonated deep soil, with middle and heavy clay texture, and high skeleton. The soil is managed with natural grass cover. The vineyard is equipped with a drip irrigation system. Plants are spaced by 2.35 m (interrow) and 1.25m (intrarow), with a plant density of about 3400 plants/ha. The training system is a double cane Guyot without spur with 12-16 winter buds/vine. Jighaura's collection rows of vineyard are directed from west to east, because of the wind direction. The management of the vineyards guarantees effective preservation of accessions and grape production.

Methods of ampelography. The OIV (2007) harmonized descriptors were used for ampelographic and ampelometric description of wild grapevine accessions, for their characteristic according to agronomic traits, resistance to biotic and abiotic stresses. The basic number of the descriptors was selected based on the COST FA1003 European Project (Appendix 1).

Ten young shoots, mature leaves, shoots, bunches and 50-100 berries of each accession were sampled and subjected to ampelographic characterization in 2019-2021 using 52 descriptors from the Organisation Internationale de la Vigne et du Vin (OIV) (2007) Vitis descriptor list.

The eno-carpological features, and the amount of polyphenols and anthocyanins were measured based on the protocols suggested by the COST FA1003 International Project (Rustioni et al. 2014b).

Phenological development of accessions were recorded using the BBCH scale (Lorenz et al. 1994) and suggested by COST FA1003 Project (Rustioni et al. 2014a).

Photos of grapevine organs were recorded in the field and in the lab.

Collected numeric data was processed by the single-dimensional statistical methods using SPSS computer software.

All ampelographic data was used for making ampelographic cards based on the model described in the book of Maghradze et al. (2017).

The results and discussions

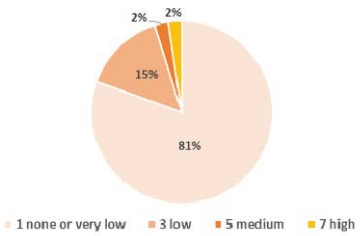
The first step of ampelographic study of the accessions available in the collection demonstrated presence of eight accessions with typical traits of cultivated grapes. These genotypes have been fragmented from this research topic and an independent group has originated.

The summarized ampelographic description of the studied accessions of *V. vinifera* ssp. *sylvestris* is provided in the Figure 2. It demonstrated that 8 descriptors are homogenous and others are heterogeneous. The homogenous ones are:

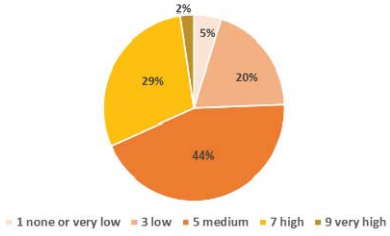
- OIV001 (Young shoot: opening of the shoot tip is fully open)
- OIV016 (Shoot: number of consecutive tendrils are two or less)
- OIV208 (Bunch: shape is cylindrical)
- OIV209 (Bunch: number of wings of the primary bunch are 1-2)
- OIV236 (Berry: particular flavor is none)
- OIV241 (Berry: formation of seeds is complete)
- OIV502 (Bunch: single bunch weight is very low)
- OIV503 (Berry: single berry weight is very low)

The OIV001 and OIV016 descriptors are the ampelographic markers for *Vitis vinifera* L. (Zdunić et al. 2017).

OIV003 - Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip

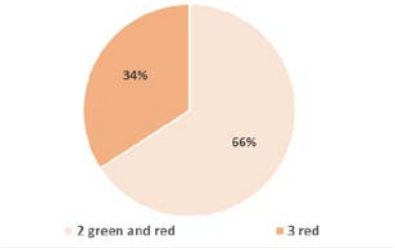


OIV004 - Young shoot: density of prostrate hairs on the shoot tip

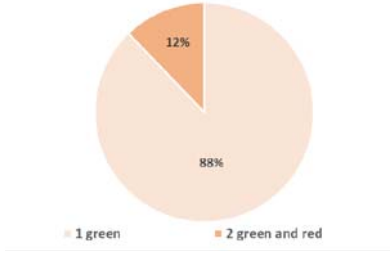


OIV006 - Shoot: attitude (before tying)

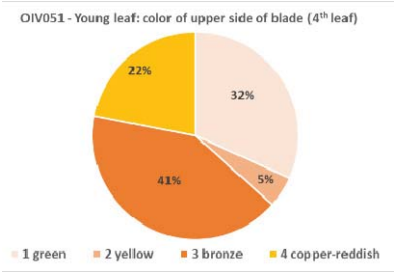
OIV007 - Shoot: color of the dorsal side of internodes



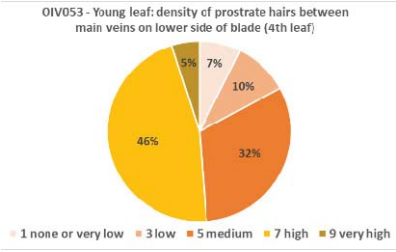
OIV008 - Shoot: color of the ventral side of internodes



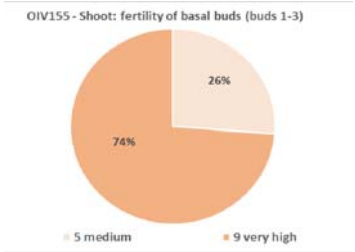
OIV051 - Young leaf: color of upper side of blade (4th leaf)



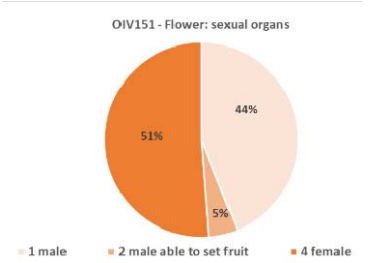
OIV053 - Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf)



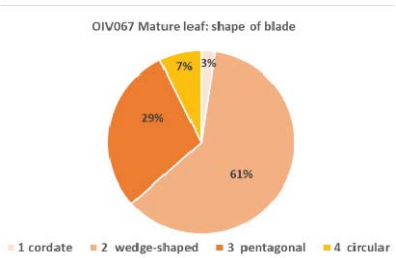
OIV155 - Shoot: fertility of basal buds (buds 1-3)



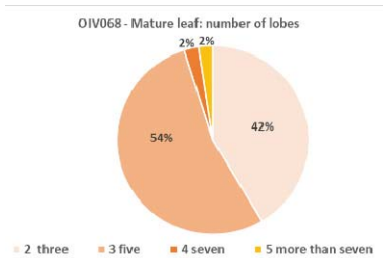
OIV151 - Flower: sexual organs



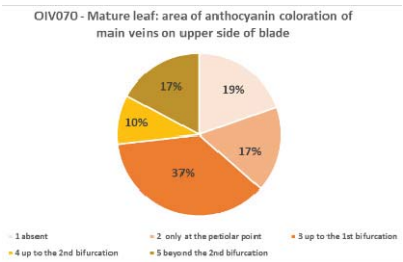
OIV067 Mature leaf: shape of blade



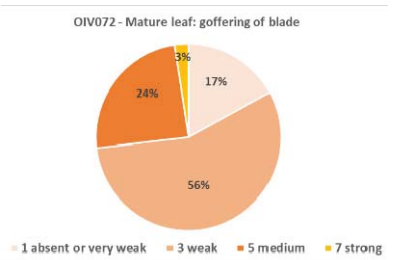
OIV068 - Mature leaf: number of lobes



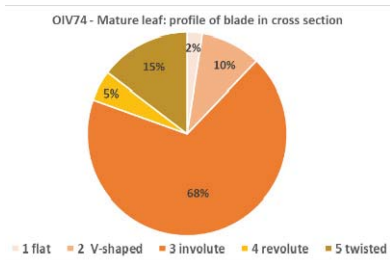
OIV070 - Mature leaf: area of anthocyanin coloration of main veins on upper side of blade



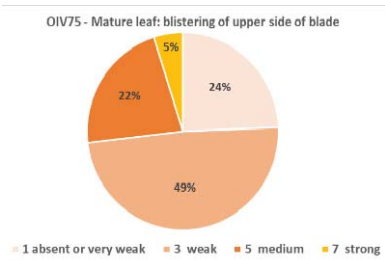
OIV072 - Mature leaf: goffering of blade



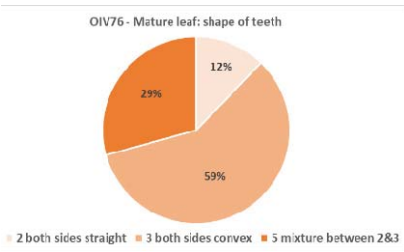
OIV74 - Mature leaf: profile of blade in cross section



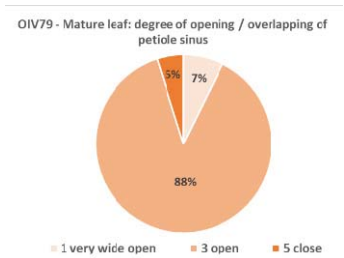
OIV75 - Mature leaf: blistering of upper side of blade



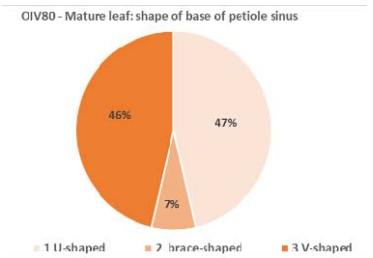
OIV76 - Mature leaf: shape of teeth



OIV79 - Mature leaf: degree of opening / overlapping of petiole sinus



OIV80 - Mature leaf: shape of base of petiole sinus



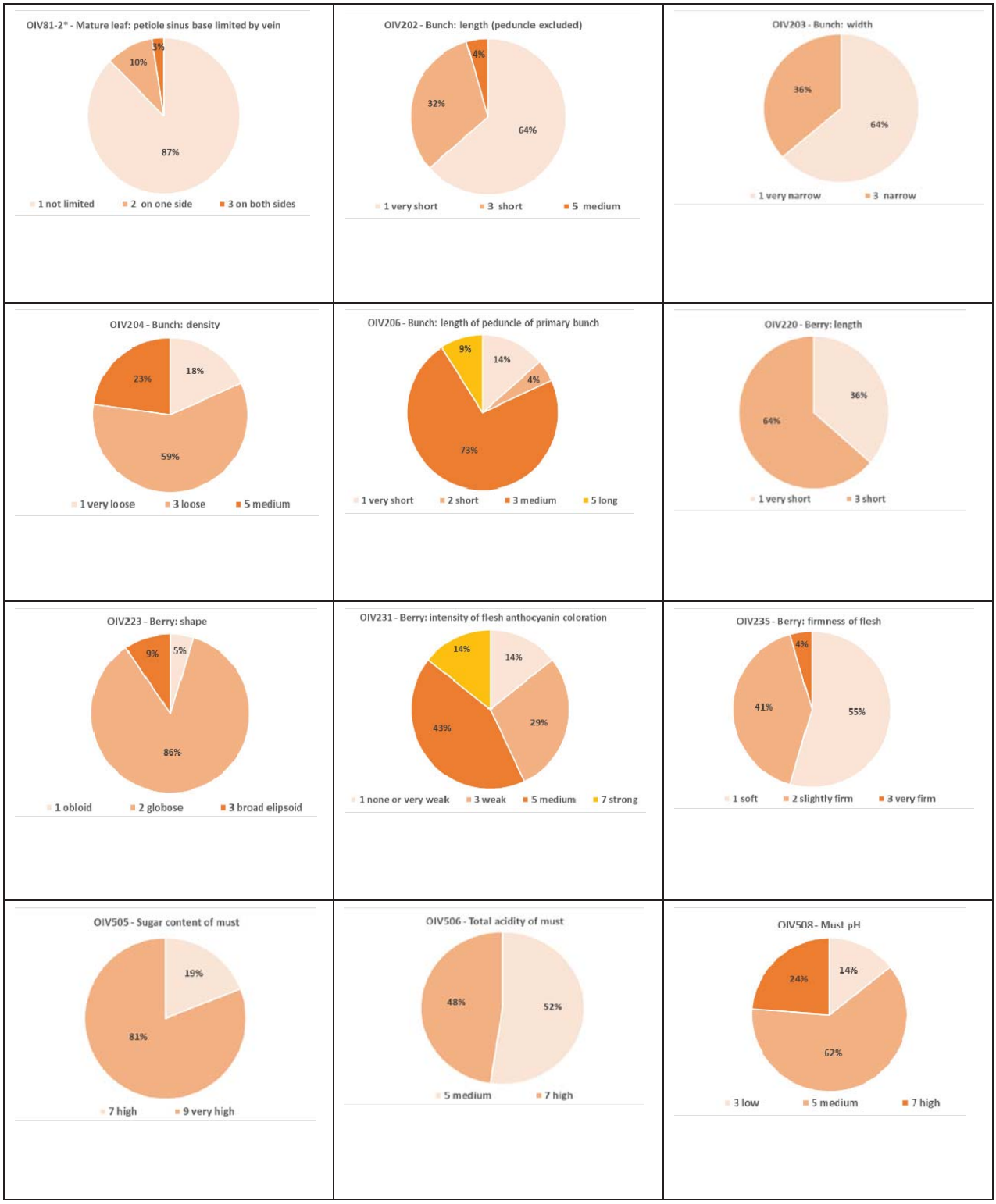


Figure 2. Polymorphic OIV descriptors

The total polyphenols and anthocyanins demonstrated wide diversity of their content in the grape: the anthocyanins varied from 438 to 2118 mg/kg in grapes and total polyphenols varied between 1187 to 3358 mg/kg in grapes (Figure 3). The control varieties Cabernet Sauvignon and Saperavi are located in the initial parts of the diagrams.

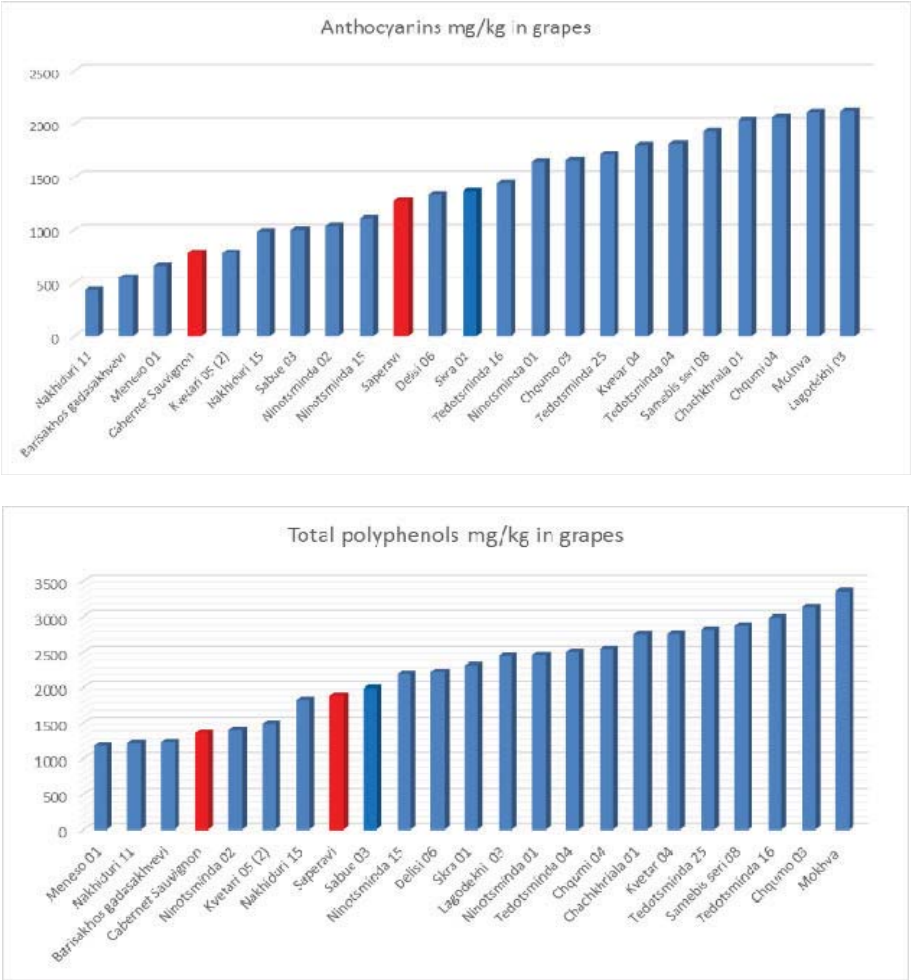


Figure 3. Total polyphenols and anthocyanins in grapes

The ampelographic cards for each studied accessions are providing in the Appendix 2.

Acknowledgments

This work was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG) FR18-18474 "Wild Grapevine of Georgia: Research and Preservation".

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Appendix 1

List of the Primary and Secondary OIV Descriptors

Selected by COST Action GRAPENET FA1003 from the 151 OIV descriptors published in June 2007

OIV	Descriptor	Note	
001	Young shoot: opening of the shoot tip	1 3 5	closed half open fully open
003	Young shoot: intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1 3 5 7 9	none or very low low medium high very high
004	Young shoot: density of prostrate hairs on the shoot tip	1 3 5 7 9	none or very low low medium high very high
006	Shoot: attitude (before tying)	1 3 5 7 9	erect semi-erect horizontal semi-drooping drooping
007	Shoot: color of the dorsal side of internodes	1 2 3	green green and red red
008	Shoot: color of the ventral side of internodes	1 2 3	green green and red red
016	Shoot: number of consecutive tendrils	1 2	2 or less 3 or more
051*	Young leaf: color of upper side of blade (4th leaf)	1 2 3 4	green yellow bronze copper-reddish
053	Young leaf: density of prostrate hairs between main veins on lower side of blade (4th leaf)	1 3 5 7 9	none or very low low medium high very high
067	Mature leaf: shape of blade	1 2 3 4 5	cordate wedge-shaped pentagonal circular kidney-shaped
068	Mature leaf: number of lobes	1 2 3 4 5	one (entire leaf) three five seven more than seven
070	Mature leaf: area of anthocyanin coloration of main veins on upper side of blade	1 2 3	absent only at the petiolar point up to the 1st bifurcation

		4 5	up to the 2nd bifurcation beyond the 2nd bifurcation
072	Mature leaf: goffering of blade	1 3 5 7 9	absent or very weak weak medium strong very strong
074	Mature leaf: profile of blade in cross section	1 2 3 4 5	flat V-shaped involute revolute twisted
075	Mature leaf: blistering of upper side of blade	1 3 5 7 9	absent or very weak weak medium strong very strong
076	Mature leaf: shape of teeth	1 2 3 4 5	both sides concave both sides straight both sides convex one side concave, one side convex mixture between both sides straight (note 2) and both sides convex (note 3)
079	Mature leaf: degree of opening / overlapping of petiole sinus	1 3 5 7 9	very wide open half open closed overlapped strongly overlapped
080	Mature leaf: shape of base of petiole sinus	1 2 3	U-shaped brace-shaped ({) V-shaped
081-1	Mature leaf: teeth in the petiole sinus	1 9	absent present
081-2*	Mature leaf: petiole sinus base limited by vein	1 2 3	not limited on one side on both sides
083-2	Mature leaf: teeth in the upper lateral sinuses	1 9	absent present
084	Mature leaf: density of prostrate hairs between main veins on lower side of blade	1 3 5 7 9	none or very low low medium high very high
087	Mature leaf: density of erect hairs on main veins on lower side of blade	1 3 5 7 9	none or very low low medium high very high
094	Mature leaf: Depth of upper lateral sinuses	1 3 5 7 9	absent or very shallow shallow medium deep very deep

103	Woody shoot: main color	1 2 3 4	yellow brownish red-violet grey
151	Flower: sexual organs	1 2 3 4	fully developed stamens and no gynoecium fully developed stamens and reduced gynoecium fully developed stamens and fully developed gynoecium reflexed stamens and fully developed gynoecium
155	Shoot: fertility of basal buds (buds 1-3)	1 5 9	very low medium very high
202	Bunch: length (peduncle excluded)	1 3 5 7 9	very short (≈80 mm) short (≈120 mm) medium (≈160 mm) long (≈200 mm) very long (≈240 mm and more)
203	Bunch: width	1 3 5 7 9	very narrow (≈40 mm) narrow (≈80 mm) medium (≈120 mm) wide (≈160 mm) very wide (≈200 mm and more)
204	Bunch: density	1 3 5 7 9	very loose loose medium dense very dense
206	Bunch: length of peduncle of primary bunch	1 3 5 7 9	very short (≈30 mm) short (≈50 mm) medium (≈70 mm) long (≈90 mm) very long (≈110 mm and more)
208	Bunch: shape	1 2 3	cylindrical conical funnel shaped
209	Bunch: number of wings of the primary bunch	1 2 3 4 5	absent 1 – 2 wings 3 – 4 wings 5 – 6 wings more than 6 wings
220*	Berry: length	1 3 5 7 9	very short (≈8 mm) short (≈13 mm) medium (≈18 mm) long (≈23 mm) very long (≈28 mm and more)
221*	Berry: width	1 3 5 7 9	very narrow (≈8 mm) narrow (≈13 mm) medium (≈18 mm) wide (≈23 mm) very wide (≈28 mm and more)

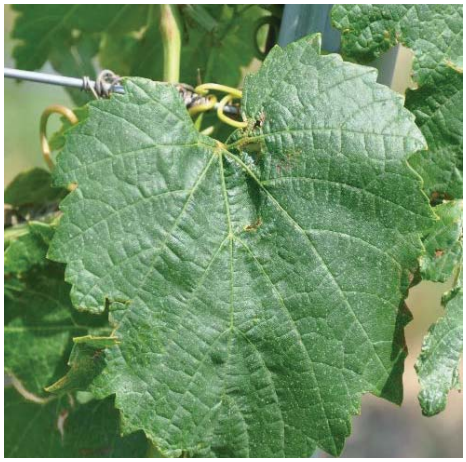
223	Berry: shape	1 2 3 4 5 6 7 8 9 10	obloid globose broad ellipsoid narrow ellipsoid cylindric obtuse ovoid ovoid obovoid horn-shaped finger-shaped
225	Berry: color of skin	1 2 3 4 5 6	green yellow rose red grey dark red violet blue black
231	Berry: intensity of flesh anthocyanin coloration	1 3 5 7 9	none or very weak weak medium strong very strong
235	Berry: firmness of flesh	1 2 3	soft slightly firm very firm
236	Berry: particular flavor	1 2 3 4 5	none muscat foxy herbaceous other flavor than muscat, foxy or herbaceous
241	Berry: formation of seeds	1 2 3	none rudimentary complete
301	Time of bud burst	1 3 5 7 9	very early early medium late very late
303	Time of beginning of berry ripening (veraison)	1 3 5 7 9	very early early medium late very late
351	Vigor of shoot growth	1 3 5 7 9	very weak weak medium strong very strong
502	Bunch: single bunch weight	1 3 5 7 9	very low (≈100 g) low (≈300 g) medium (≈500 g) high (≈700 g) very high (≈800 g and more)

503	Berry: single berry weight	1 3 5 7 9	very low (≈1 g) low (≈3 g) medium (≈5 g) high (≈7 g) very high (≈9 g and more)
504	Yield per m^2	1 3 5 7 9	very low (≈0.5 kg) low (≈0.5 - 0.8 kg) medium (≈0.8 - 1.0 kg) high (≈1.2 - 1.5 kg) very high (≈1.5 kg and more)
505	Sugar content of must	1 3 5 7 9	very low (≈12%) low (≈15%) medium (≈18%) high (≈21%) very high (≈24%)
506	Total acidity of must: Tartaric acid	1 3 5 7 9	very low (≈3 g/l) low (≈6 g/l) medium (≈9 g/l) high (≈12 g/l) very high (≈15 g/l)
508	Must specific pH	3 5 7	low (<3.0) medium low (<3.0-3.3) high low (>3.3)

Optional

452	Leaf*:* degree of resistance to ***Plasmopara***	1 3 5 7 9	very low low medium high very high or total
452-1	Leaf*:* degree of resistance to ***Plasmopara*** (leaf disc test)	1 3 5 7 9	very low low medium high very high or total

Asureti 01



Passport information	
Number in collection	GEO038-W2014-021
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Asureti is a village of Tetritskaro district of Lower Kartli, where the plant was descovered.

Historical facts and distribution

The plant was detected in the North slop of river Asuretiskhevi – the left tributary of Algeti river - in 2004. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	3	low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf:			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Bliste ring of upper side of blade	1	absent or very weak
OIV076	Shape of teeth	2	both sides straight
OIV079	Degree of opening / overlapping of petiole sinus	3	closed
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	none
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	none
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	1	shallow

Asureti 01

Flower:			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

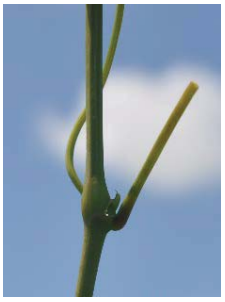
Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	me dium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phebotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.





Bagichala 04/05

Passport information	
Number in collection	GEO038-W2014-007
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Bagichala is name of the river in Dusheti district of East Georgia, where the plants was discovered.

Historical facts and distribution

The plant was detected in the Aragvi river basin in 2004. It grew in the North slope. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lower side of blade	3	low
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	1	absent
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	2	V-shaped
OIV075	Blistering of upper side of blade	5	medium
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	2	open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	3	on both sides
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	1	absent or very shallow

Bagichala 04/05

Flower:			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
ბუჩქი			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	3	low

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	29 May
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

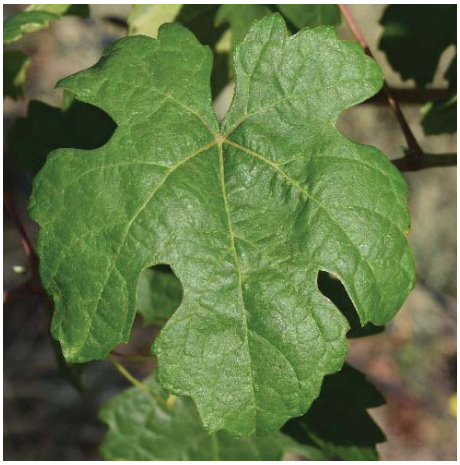
No grapes are available.







Bagichala 07



Passport information	
Number in collection	GEO038-W2014-008
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Bagichala is a name of the river in Dusheti district of East Georgia, where the plants was discovered.

Historical facts and distribution

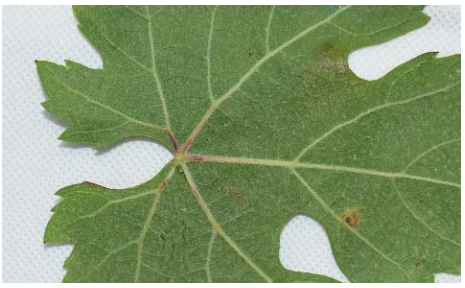
The plant was detected in the Aragvi river basin in 2004. It grew in the North slope. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf:			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	2	only at the petiolar point
OIV072	Goffering of blade	5	medium
OIV074	Profile of blade in cross section	3	involute
OIV075	Bliste ring of upper side of blade	5	medium
OIV076	Shape of teeth	5	mixture between both sides straight (note 2) and both sides convex (note 3)
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V - shape
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	7	deep

Bagichala 07

Flower:			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	–	–
OIV203	Width	–	–
OIV204	Density	–	–
OIV206	Length of peduncle of primary bunch	–	–
OIV208	Shape	–	–
OIV209	Number of wings of the primary bunch	–	–
Berry			
OIV220	Length	–	–
OIV221	Width	–	–
OIV223	Shape	–	–
OIV225	Color of skin	–	–
OIV231	Intensity of flesh anthocyanin coloration	–	–
OIV235	Firmness of flesh	–	–
OIV236	Particular flavor	–	–
OIV241	Formation of seeds	–	–
Elements of productivity			
OIV502	Single bunch weight	–	–
OIV503	Single berry weight	–	–
OIV504	Harvest m^2	–	–
Characteristics of grape juice			
OIV505	Sugar content of must	–	–
OIV506	Total acidity of must	–	–
OIV508	Must specific pH	–	



Degree of resistance to Plasmopara	
OIV432-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test) 7 high

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	29 May
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.

Bagichala 12

Passport information	
Number in collection	GEO038-W2014-010
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Bagichala is a name of the river in Dusheti district of East Georgia, where the plants was discovered.

Historical facts and distribution

The plant was detected in the Aragvi river basin in 2004. It grew in the North slope. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	3	low
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	5	medium
Young (4th) leaf:			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	2	only at the petiolar point
OIV072	Goffering of blade	5	medium
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	2	both sides straight
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	3	shallow

Bagichala 12

Flower:			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

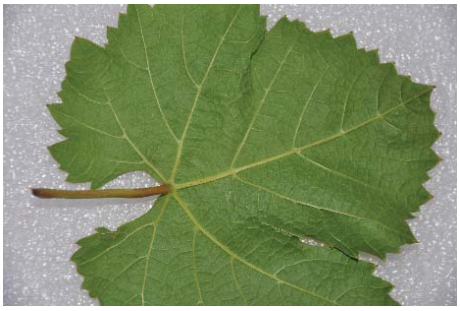
Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.



Barisakhos Gadasakhvevi

Passport information	
Number in collection	GEO038-W2014-031
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Barisakhos Gadasakhvevi is a place in Dusheti distrct of Estern Georgia where the plants was descovered.

Historical facts and distribution

The plant was de tected in Dusheti district in 2005. It grew in North-Est slop of Aragvi river gorge. Now it is preserved in Jighaura collection

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	7	high
OIV004	Density of prostrate hairs on the shoot tip	3	low
Shoot			
OIV006	Attitude (before tying)	5	horizontal
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	2	green and red
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf:			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	1	absent or very weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	5	mixture between both sides straight and both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	open
OIV080	Shape of base of petiole sinus	2	brace-shaped ({)
OIV081-1	Teeth in the petiole sinus	1	none
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	5	medium

Barisakhos Gadasakhvevi

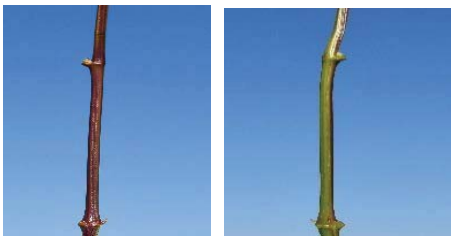
Flower:			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1	very short
OIV203	Width	1	very narrow
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 - 2 wings
Berry			
OIV220	Length	1	very short
OIV221	Width	1	very narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	1	none or very weak
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	7	high
OIV508	Must specific pH	5	medium

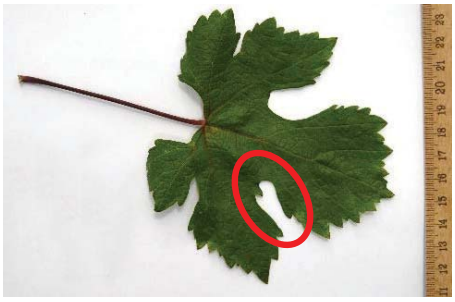
Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	**mg / kg in grapes**
Total anthocyanins	553
Total polyphenols	1237

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	8 August
-	Berries ripe for harvest	23 September

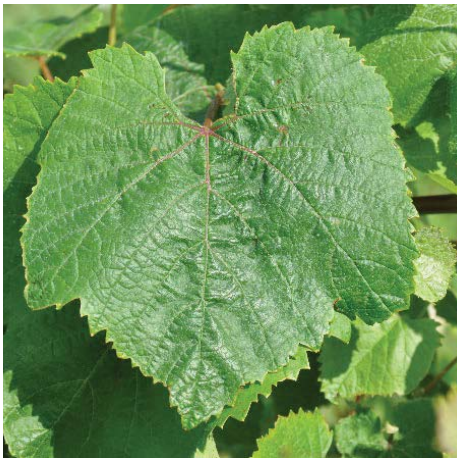
Characterization of wine and grapes-
The bunch is small, sparse, with small black berries. The must has high sugar and acidity content.





Chachkhriala 01



Passport information	
Number in collection	GEO038-W2014-034
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Chachkhriala is a village of Akmeta district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in North-Estern slope of Ilto river, the right tributary of Alazani river basin, in 2006. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitu de (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	4	copper-reddish
OIV053	Density of prostrate hairs between main veins on lowerside of blade	7	high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	5	beyond the 2nd bifurcation
OIV072	Goffering of blade	5	medium
OIV074	Profile of blade in cross section	3	involute
OIV075	Bliste ring of upper side of blade	3	weak
OIV076	Shape of teeth	5	mixture between both sides straight (note 2) and both sides convex (note 3)
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	3	shallow

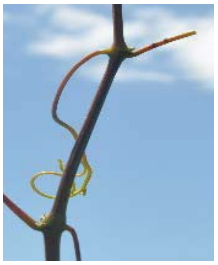
Chachkhriala 01

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1	very short
OIV203	Width	1	very narrow
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	1	very short
OIV221	Width	1	very narrow
OIV223	Shape	1	obloid
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	3	weak
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	7	high
OIV508	Must specific pH	5	medium

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	2035
Total polyphenols	2747

Phenotyping trial		
OIV301	Time of bud burst	14 April
-	Beginning of flowering	4 June
OIV303	Time of beginning of berry ripening (veraison)	6 August
-	Berries ripe for harvest	7 September






Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has high sugar and medium acidity content.

Chqumi 02

Passport informat ion	
Number in collection	GEO038-W2014-049
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Chqumi is a village of Tsageri district of Lechkhumi, where the plant was descovered.

Historical facts and distribution

The plant was detected in the Tskhenistskali river basin in 2007. It grew in the North-Estern slope. Recently the plants is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	3	low
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitu de (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	4	copper-reddish
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	5	beyond the 2nd bifurcation
OIV072	Goffering of blade	5	medium
OIV074	Profile of blade in cross section	5	twisted
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	5	mixture between both sides straight (note 2) and both sides convex (note 3)
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	1	absent or very shallow

Chqumi 02

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.

Chqumi 03

Passport information	
Number in collection	GEO038-W2014-052
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Chqumi is a village of Tsageri district of Lechkhumi, where the plant was descovered.

Historical facts and distribution

The plant was d etected in the North-Estern banck of river Jonoula – the right tributary of Tskhenistskali river - in 2007. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lowerside of blade	9	very high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	5	beyond the 2nd bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	1	flat
OIV075	Bliste ring of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	1	absent or very shallow

Chqumi 03

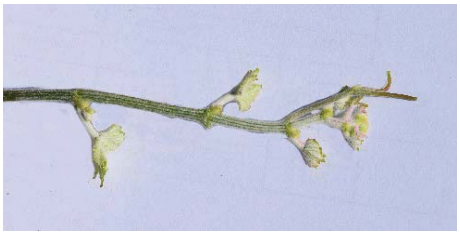
Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1	very short
OIV203	Width	1	very narrow
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	3	broad ellipsoid
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	7	strong
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	7	high
OIV508	Must specific pH	3	low

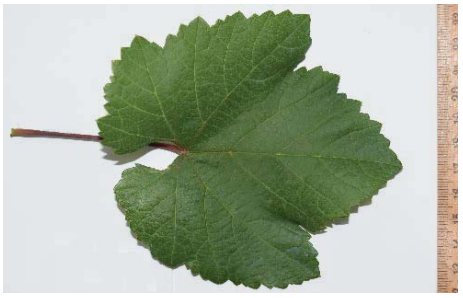
Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	3	low

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	1656
Total polyphenols	3129

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	10 August
-	Berries ripe for harvest	7 September





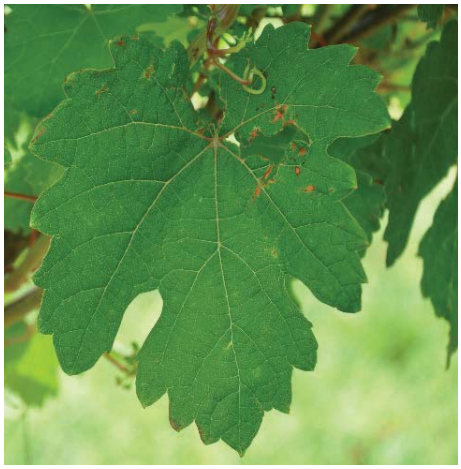




Characterization of wine and grapes-

The bunch is small, sparse, with small black berries. The must has high sugar and acidity content.

Chqumi 04



Passport information	
Number in collection	GEO038-W2014-040
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Chqumi is a village of Tsageri district of Lechkhumi, where the plant was descovered.

Historical facts and distribution

The plant was d etected in the North-Estern banck of river Jonoula – the right tributary of Tskhenistskali river - in 2007. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	1	none or very low
Shoot			
OIV006	Attitu de (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	2	green and red
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	1	absent or very weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	5	medium

Chqumi 04

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	3	short
OIV203	Width	3	narrow
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	1	obloid
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	7	strong
OIV235	Firmness of flesh	2	slightly firm
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	7	high
OIV508	Must specific pH	5	medium

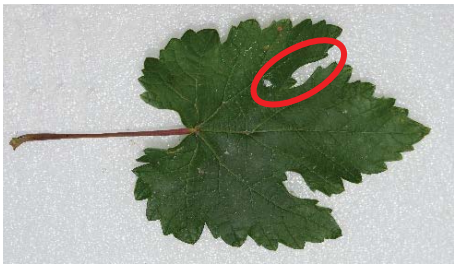
Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	2064
Total polyphenols	2545

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	16 August
-	Berries ripe for harvest	15 September










Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has high sugar and acidity content.

Chqumi 06

Passport information	
Number in collection	GEO038-W2014-045
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Chqumi is a village of Tsageri district of Lechkhumi, where the plant was descovered.

Historical facts and distribution

The plant was detected in the North-Estern banck of river Jonoula – the right tributary of Tskhenistskali river - in 2007. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lowerside of blade	5	medium
Mature leaf			
OIV067	Shape of blade	4	circular
OIV068	Numb er of lobes	5	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	5	beyond the 2nd bifurcation
OIV072	Goffering of blade	1	absent or very weak
OIV074	Profile of blade in cross section	5	twisted
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	3	shallow

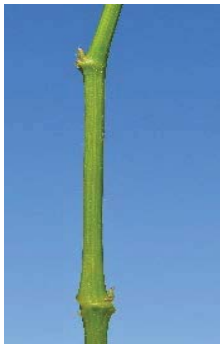
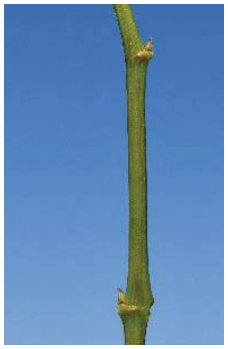
Chqumi 06

Flower			
OIV151	Sexual organs	2	fully developed stamens and reduced gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	3	low

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	3 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-




Characterization of wine and grapes

The bunch is small, sparse, with small black berries.

Delisi 06

Passport information	
Number in collection	GEO038-W2014-009
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Delisi is a name of locality close to Tbilisi, where the plant was descovered.

Historical facts and distribution

The plant was detected in the river basin of Mtkvari (Kura) - in 2008. It is grew on the South - directrd slop. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf:			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	1	absent or very weak
OIV074	Profil e of blade in cross section	2	V-shaped
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	3	shallow

Delisi 06

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1	very short
OIV203	Width	1	very narrow
OIV204	Density	1	very loose
OIV206	Length of peduncle of primary bunch	3/5	short /medium
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	5	medium
OIV235	Firmness of flesh	2	slightly firm
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m²	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	5	medium
OIV508	Must specific pH	7	high

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	1336
Total polyphenols	2223

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	10 August
-	Berries ripe for harvest	11 September









Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has high sugar and acidity content

Enageti 01

Passport information	
Number in collection	GEO038-W2014-033
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Enageti is a village of Tetritskaro district of Lower Kartli, where the plant was descovered.

Historical facts and distribution

The plant was detected in the South slop of river Enagetiskhevi – the left tributary of Algeti river - in 2004. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lower side of blade	9	very high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	1	absent
OIV072	Goffering of blade	5	medium
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	1	absent or very weak
OIV076	Shape of teeth	5	mixture between both sides straight (note 2) and both sides convex (note 3)
OIV079	Degree of opening / overlapping of petiole sinus	5	closed
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	2	on one side
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	5	medium
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	3/5	shallow /medium

Enageti 01

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	7	high

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.







Kvetari 04

Passport information	
Number in collection	GEO038-W2014-050
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi (?)
Country	Georgia

Synonyms, meaning of name

Kvetari is name of a historical early medieval town and fortification, locatedin Akmeta district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in North slope of Ilto river, the right tributary of Alazani river basin, in 2006. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	3	low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitu de (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	2	green and red
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	5	beyond the 2nd bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	5	twisted
OIV075	Bliste ring of upper side of blade	3	weak
OIV076	Shape of teeth	5	mixture between both sides straight (note 2) and both sides convex (note 3)
OIV079	Degree of opening / overlapping of petiole sinus	1	very wide open
OIV080	Shape of base of petiole sinus	2	brace-shaped ({)
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	3	shallow

Kvetari 04

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	3	short
OIV203	Width	1	very narrow
OIV204	Density	5	medium
OIV206	Length of peduncle of primary bunch	1	very short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	1	very short
OIV221	Width	1	very narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	7	strong
OIV235	Firmness of flesh	2	slightly firm
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	5	medium
OIV508	Must specific pH	5	medium

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	1802
Total polyphenols	2754

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	8 August
-	Berries ripe for harvest	9 September









Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has high sugar and acidity content.

Kvetari 05 (2)

Passport information	
Number in collection	GEO038-W2014-044
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Kvetari is name of a historical early medieval town and fortification, located in Akmeta district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in North slope of Ilto river, the right tributary of Alazani river b asin, in 2006. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	4	copper-reddish
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	5	beyond the 2nd bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	5	twisted
OIV075	Bliste ring of upper side of blade	5	medium
OIV076	Shape of teeth	5	mixture between both sides straight (note 2) and both sides convex (note 3)
OIV079	Degree of opening / overlapping of petiole sinus	1	open
OIV080	Shape of base of petiole sinus	2	brace-shaped ({)
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	5	medium
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	5	medium

Kvetari 05 (2)

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1	very short
OIV203	Width	1	very narrow
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	1	very short
OIV221	Width	1	very narrow
OIV223	Shape	3	broad ellipsoid
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	5	medium
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	7	high
OIV506	Total acidity of must	5	medium
OIV508	Must specific pH	5	medium low

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	7	high

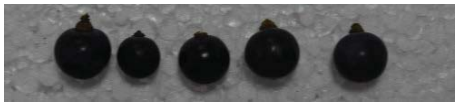
Biochemical characteristics	mg / kg in grapes
Total anthocyanins	785
Total polyphenols	1488

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	9 August
-	Berries ripe for harvest	15 September









Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has high sugar and acidity content.

Lagodekhi (the 60th quarter) 03

Passport information	
Number in collection	GEO038-W2014-013
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Lagodekhi is name of wild grapes population, descoverd in Lagodeghi reserve, Kakheti.

Historical facts and distribution

The plant was detected in South slope, not far from Matsimisstkali river, the right tributary of Alazani river basin, in 2013. Recently the plant is preservedin Jighaura collect ion..

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	4	copper-reddish
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	4	circular
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	5	beyond the 2nd bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	1	very wide open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	2	on one side
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	3	shallow

Lagodekhi (the 60th quarter) 03

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	3	short
OIV203	Width	1	very narrow
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	1	very narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	5	medium
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	7	high
OIV508	Must specific pH	5	medium

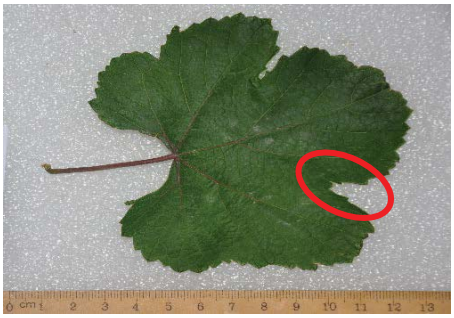
Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	2118
Total polyphenols	2445

Phenotyping trial		
OIV301	Time of bud burst	21 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	9 August
-	Berries ripe for harvest	11 September









Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has high sugar and medium acidity content

Meneso 01

Passport information	
Number in collection	GEO038-W2014-011
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Meneso is name of a village in Dusheti district of East Georgia, where the plant was desvovered.

Historical facts and distribution

The plant was detected in North slope of Aragvi river in 2004. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Num ber of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	2	only at the petiolar point
OIV072	Goffering of blade	1	absent or very weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Bliste ring of upper side of blade	3	weak
OIV076	Shape of teeth	5	mixture between both sides straight (note 2) and both sides convex (note 3)
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	2	on one side
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	5	medium

Meneso 01

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1/3	very short /short
OIV203	Width	1	very narrow
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blu e black
OIV231	Intensity of flesh anthocyanin coloration	3	weak
OIV235	Firmness of flesh	2	slightly firm
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	7	high
OIV508	Must specific pH	5	medium

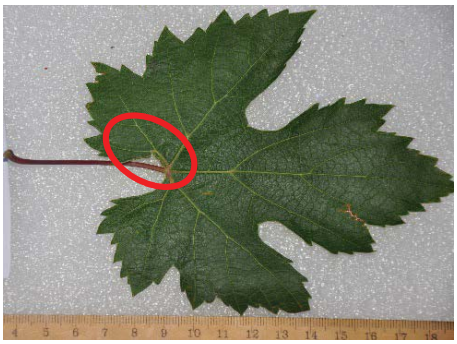
Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	643
Total polyphenols	1187

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	1 June
OIV303	Time of beginning of berry ripening (veraison)	10 August
-	Berries ripe for harvest	11 September





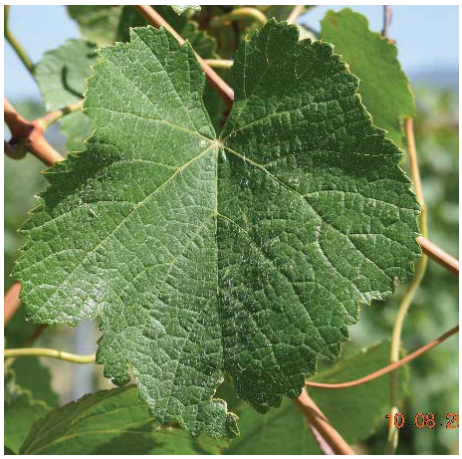




Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has high sugar and acidity content.

Mokhva



Passport information	
Number in collection	GEO038-W2014-048
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Mokhva is a village of Sachkhere district in Imereti, where the plant was descovered.

Historical facts and distribution

The plant was detected in the North slop of river Jruchula – the right tributary of Kvirila river - in 2006. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	2	green and red
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	1	absent
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	1	absent or very weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	5	medium
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	1	absent or very shallow

Mokhva

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1	very short
OIV203	Width	1	very narrow
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	5	medium
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	5	medium
OIV508	Must specific pH	3	low

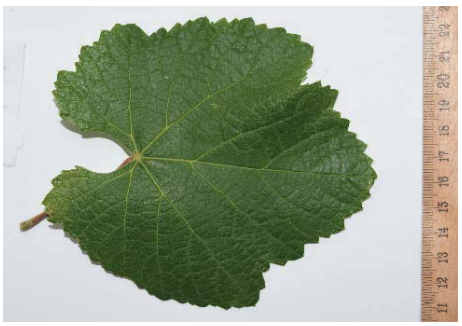
Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	7	high

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	2109
Total polyphenols	3358

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	9 August
-	Berries ripe for harvest	17 September









Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has high sugar and acidity content.

Nakhiduri 02

Passport information	
Number in collection	GEO038-W2014-018
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Nakhiduri is a village of Bolnisi district of Lower Kartli, where the plant was descovered.

Historical facts and distribution

The plant was detected in the right bank of river Ktsia-Khrami, on the North-directed slop in 2005. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	3	low
OIV004	Density of prostrate hairs on the shoot tip	3	low
Shoot			
OIV006	Attitude (before tying)	5	horizontal
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	3/4	Pentagonal /circular
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	5	medium
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	5	medium

Nakhiduri 02

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

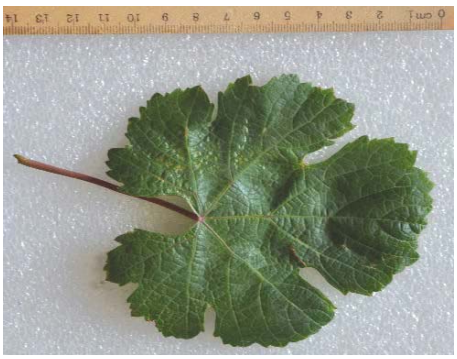
Phenotyping trial		
OIV301	Time of bud burst	21 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

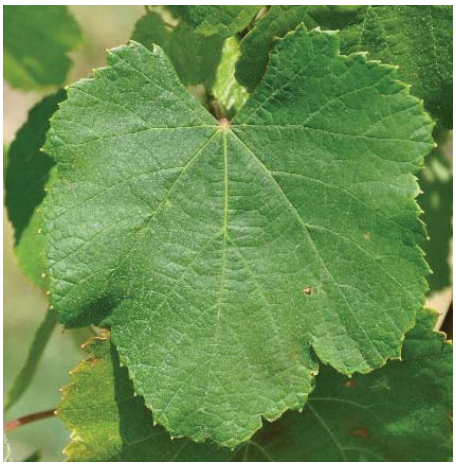
No grapes are available.






Nakhiduri 11



Passport information	
Number in collection	GEO038-W2014-017
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Nakhiduri is a village of Bolnisi district of Lower Kartli, where the plant was descovered.

Historical facts and distribution

The plant was detected in the right bank of river Ktsia-Khrami, on the North-directed slop in 2013. Recently the plant is preserved in Jighaura collection.

Basic ampelograp hic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	5	horizontal
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	5	medium
Young (4th) leaf			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	3	shallow

Nakhiduri 11

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded	3	short
OIV203	Width	3	narrow
OIV204	Density	5	medium
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	3	weak
OIV235	Firmness of flesh	2	slightly firm
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	7	high
OIV508	Must specific pH	5	medium

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	**mg / kg in grapes**
Total anthocyanins	438
Total polyphenols	1223

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	18 August
-	Berries ripe for harvest	11 September





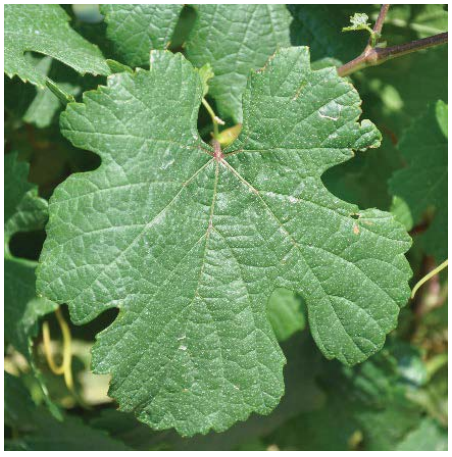




Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has high sugar and acid content

Nakhiduri 15



Passport information	
Number in collection	GEO038-W2014-014
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Nakhiduri is a village of Bolnisi district of Lower Kartli, where the plant was descovered.

Historical facts and distribution

The plant was detected in the right bank of river Ktsia-Khrami, on the North-directed slop in 2013. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	1	none or very low
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	5	medium
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	4	up to the 2nd bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	5	medium

Nakhiduri 15

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	3	short
OIV203	Width	1	very narrow
OIV204	Density	5	medium
OIV206	Length of peduncle of primary bunch	1/3	very short / short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	1	none or very weak
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	7	high
OIV506	Total acidity of must	7	high
OIV508	Must specific pH	3	low

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	979
Total polyphenols	1827

Phenotyping trial		
OIV301	Time of bud burst	21 April
-	Beginning of flowering	1 June
OIV303	Time of beginning of berry ripening (veraison)	10 August
-	Berries ripe for harvest	11 September









Characterization of wine and grapes

The bunch is small, spares and has small black berries. The juice has high sugar and acid content.

Ninotsminda 01

Passport information	
Number in collection	GEO038-W2014-005
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Ninotsminda is a village of Sagarejo district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in the South slop of Gombori mountail range in 2006, belonging Iori river basin. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	3	low
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	5	medium
Young (4th) leaf			
OIV051	Color of upper side of blade	4	copper-reddish
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	4	seven
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	2	only at the petiolar point
OIV072	Goffering of blade	5	medium
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	5	medium

Ninotsminda 01

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded	1	very short
OIV203	Width	3	narrow
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	5	medium
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	3	weak
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	7	high
OIV506	Total acidity of must	5	medium
OIV508	Must specific pH	7	high

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	1641
Total polyphenols	2456

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	9 August
-	Berries ripe for harvest	8 September







Characterization of wine and grapes

The bunch is small, sparse, with small black and globose berries. The must has high sugar and medium acid content.

Ninotsminda 02

Passport information	
Number in collection	GEO038-W2014-004
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Ninotsminda is a village of Sagarejo district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in the South slop of Gombori mountail range in 2006, belonging Iori river basin. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	3	low
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	5	medium
Young (4th) leaf			
OIV051	Color of upper side of blade	4	copper-reddish
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	4	revolute
OIV075	Blistering of upper side of blade	5	medium
OIV076	Shape of teeth	2	both sides straight
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	2	on both sides
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	5	medium
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	5	medium

Ninotsminda 02

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1/3	very short /short
OIV203	Width	3	narrow
OIV204	Density	5	medium
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	3	weak
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	5	medium
OIV508	Must specific pH	7	high low

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

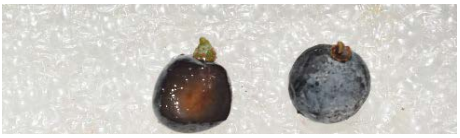
Biochemical characteristics	mg / kg in grapes
Total anthocyanins	1043
Total polyphenols	1397

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	9 August
-	Berries ripe for harvest	6 September










Characterization of wine and grapes

The bunch is small, sparse, with small black and globose berries. The must has very high sugar and medium acidity content.

Ninotsminda 06/07

Passport information	
Number in collection	GEO038-W2014-006
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Ninotsminda is a village of Sagarejo district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in the South slop of Gombori mountail range in 2006, belonging Iori river basin. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	5	medium
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	2	only at the petiolar point
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	5	twisted
OIV075	Blistering of upper side of blade	5	medium
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	5	closed
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	7	high
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	1	absent or very shallow

Ninotsminda 06/07

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	4	
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	15 April
-	Beginning of flowering	1 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

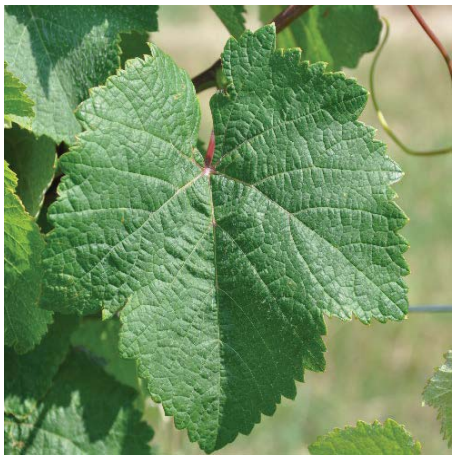
No grapes are available.







Ninotsminda 11



Passport information	
Number in collection	GEO038-W2014-003
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Ninotsminda is a village of Sagarejo district of Kakheti, where the plant was descovered.

Historical facts a and distribution

The plant was detected in the South slop of Gombori mountail range in 2006, belonging Iori river basin. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	5	medium
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	5	medium
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	7	strong
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	5	medium
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	3	shallow

Ninotsminda 11

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.

Ninotsminda 15

Passport information	
Number in collection	GEO038-W2014-002
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Ninotsminda is a village of Sagarejo district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in the South slop of Gombori mountail range in 2006, belonging Iori river basin. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	5	horizontal
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	5	medium
Young (4th) leaf			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Numb er of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	4	beyond the 2nd bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	2	V-shaped
OIV075	Bliste ring of upper side of blade	7	strong
OIV076	Shape of teeth	2	both sides straight
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	5	medium
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	5	medium

Ninotsminda 15

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1	very short
OIV203	Width	1	very narrow
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	1	very short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	5	medium
OIV235	Firmness of flesh	2	slightly firm
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	5	medium
OIV508	Must specific pH	5	medium

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	1111
Total polyphenols	2196

Phenotyping trial		
OIV301	Time of bud burst	14 April
-	Beginning of flowering	1 June
OIV303	Time of beginning of berry ripening (veraison)	9 August
-	Berries ripe for harvest	11 September

Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has very high sugar and medium acidity content.








Sabue 01

Passport information	
Number in collection	GEO038-W2014-051
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Sabue is a village of Akmeta district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in the banck of Ilto river, the right tributary of Alazani river basin, in 2006. The plant grew in North slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	2	yellow green
OIV053	Density of prostrate hairs between main veins on lower side of blade	3	low
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	1	absent
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	5	medium
OIV076	Shape of teeth	5	mixture between both sides straight (note 2) and both sides convex (note 3)
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	5	medium

Sabue 01

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

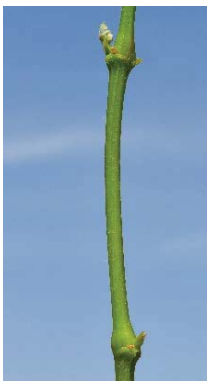
Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	7	high

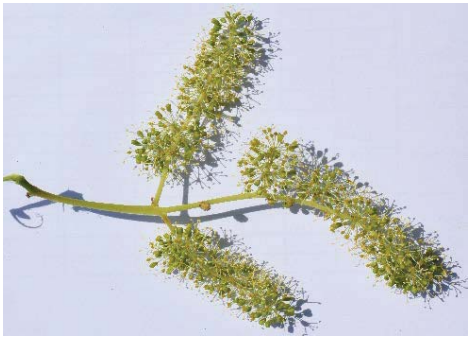
Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

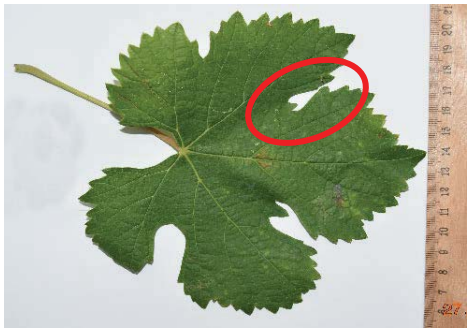
Phenotyping trial		
OIV301	Time of bud burst	15 April
-	Beginning of flowering	1 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.






Sabue 03

Passport information	
Number in collection	GEO038-W2014-020
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Sabue is a village of Akmeta district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in the banck of Ilto river, the right tributary of Alazani river basin, in 2006. The plant grew in North slope. Recently it is preserved in Jig haura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinu	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	1	absent or very shallow

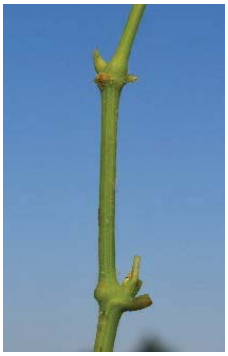
Sabue 03

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1	very short
OIV203	Width	1	
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	1	very short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	5	medium
OIV235	Firmness of flesh	2	slightly firm
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	5	medium
OIV508	Must specific pH	5	medium

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	-	-

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	995
Total polyphenols	1993

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	1 June
OIV303	Time of beginning of berry ripening (veraison)	14 August
-	Berries ripe for harvest	7 September



Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has very high sugar and medium acidity content.

Samebis seri 08

Passport information	
Number in collection	GEO038-W2014-012
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Samebis seri is a name of the hill close to town Kvareli in Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected close of the bank of Duruji river, the left tributary of Alazani river basin, in 2006. The plant grew in South slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	3	low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	4	copper-reddish
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	3	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	7	strong
OIV074	Profile of blade in cross section	4	revolute
OIV075	Blistering of upper side of blade	5	medium
OIV076	Shape of teeth	5	mixture between both sides straight (note 2) and both sides convex (note 3)
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	1	absent or very shallow

Samebis seri 08

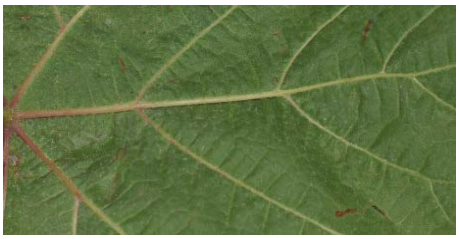
Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1	very short
OIV203	Width	1	very narrow
OIV204	Density	3	Loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	5	medium
OIV235	Firmness of flesh	2	slightly firm
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	7	high
OIV508	Must specific pH	5	medium

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	3	low

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	1928
Total polyphenols	2873

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	1 June
OIV303	Time of beginning of berry ripening (veraison)	8 August
-	Berries ripe for harvest	11 September



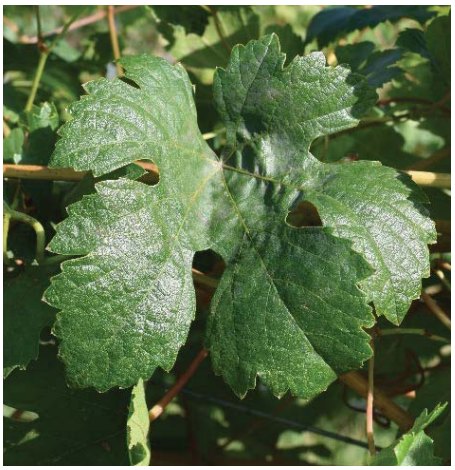






Characterization of wine and grapes

The bunch is small, sparse, with small black and globose berries. The must has very high sugar and medium acid content.

Sartichala (ferma) 02



Passport information	
Number in collection	GEO038-W2014-042
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Sartichala (ferma) is a name of the locality in Sagarejo district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in on of tributary of Iori river basin, in 2011. The plant grew in North-West slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	4	seven
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	2	only at the petiolar point
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	2	V-shaped
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	3	on both sides
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	7	deep

Sartichala (ferma) 02

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	-	-

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	20 April
-	Beginning of flowering	3 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.

Sartichala (ferma) 07

Passport information	
Number in collection	GEO038-W2014-039
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Sartichala (ferma) is a name of the locality in Sagarejo district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in on of tributary of Iori river basin, in 2011. The plant grew in North-directed slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	3	low
Shoot			
OIV006	Attitude (before tying)	5	horizontal
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	1	none or very low
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	1	absent or very weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	5	medium

Sartichala (ferma) 07

Flower			
OIV151	Sexual organs	2	fully developed stamens and reduced gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	3	sho rt
OIV203	Width	3	narrow
OIV204	Density	1	very loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	1	ver y short
OIV221	Width	1	very narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

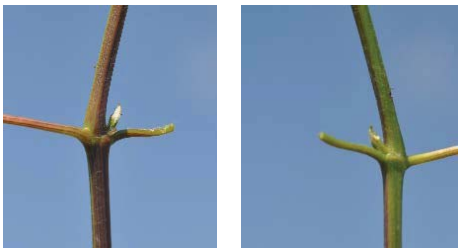
Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

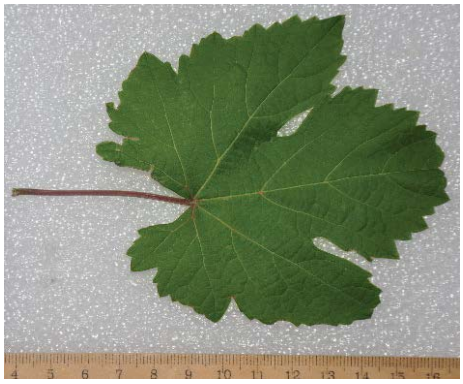
Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	11 august
-	Berries ripe for harvest	27 September

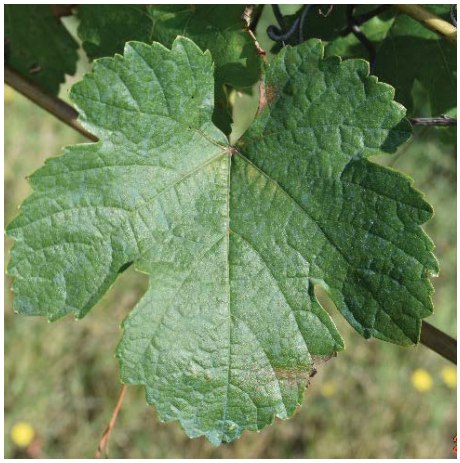
Characterization of wine and grapes

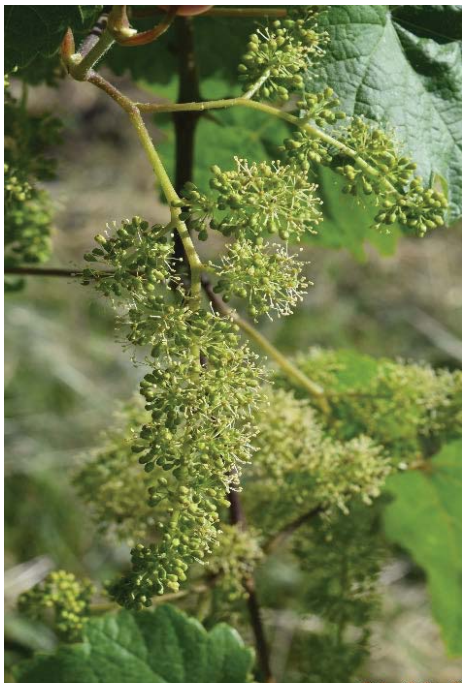
The bunch is small, very sparse, with small black berries.





Sartichala (ferma) 11





Passport information	
Number in collection	GEO038-W2014-041
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Sartichala (ferma) is a name of the locality in Sagarejo district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in on of tributary of Iori river basin, in 2011. The plant grew in North-directed slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	3	low
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	4	up to the 2nd bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	5	twisted
OIV075	Blistering of upper side of blade	5	medium
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	3	shallow

Sartichala (ferma) 11

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.







Shirikhevi 03

Passport information	
Number in collection	GEO038-W2014-036
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Shirikhevi is a name of the river in Dusheti district of East Georgia, where the plants was discovered.

Historical facts and distribution

The plant was detected in the bank of Shirikhevi river, the right tributary of Aragvi river basin, in 2004. It grew in the North slope. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	3	red
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	4	up to the 2nd bifurcation
OIV072	Goffering of blade	1	absent or very weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	3	shallow

Shirikhevi 03

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

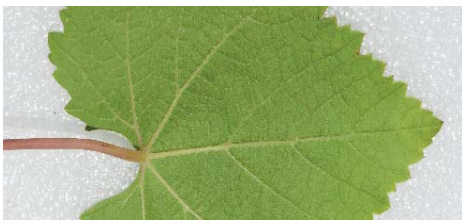
Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Totally anthocyanins	-
Totally polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.



Shirikhevi 04

Passport information	
Number in collection	GEO038-W2014-037
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Shirikhevi is a name of the river in Dusheti district of East Georgia, where the plants was discovered.

Historical facts and distribution

The plant was detected in the bank of Shirikhevi river, the right tributary of Aragvi river basin, in 2004. It grew in the North slope. Recently the plant is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	2	only at the petiolar point
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	1	absent or very weak
OIV076	Shape of teeth	2	both sides straight
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	5	medium
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	3	shallow

Shirikhevi 04

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.

Skra 01

Passport information	
Number in collection	GEO038-W2014-032
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Skra is a village of Gori district of Inner Kartli, where the plant was descovered.

Historical facts and distribution

The plant was detected in the banck of Mtkvari (Kura) river, in 2013. The plant grew in North-directed slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	9	very high
Shoot			
OIV006	Attitude (before tying)	5	horizontal
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	2	Red and green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	5	medium
Young (4th) leaf			
OIV051	Color of upper side of blade	2	yellow green
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	1	absent or very weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	3	shallow

Skra 01

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	4	gre y
Bunch			
OIV202	Length (peduncle excluded	1	ver y short
OIV203	Width	3	narrow
OIV204	Density	3	loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	1	very short
OIV221	Width	1	very narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	3	weak
OIV235	Firmness of flesh	3	very firm
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	5	medium
OIV508	Must specific pH	5	medium

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	7	high

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	1366
Total polyphenols	2316

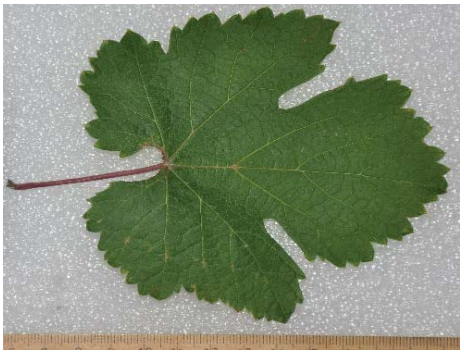
Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	1 June
OIV303	Time of beginning of berry ripening (veraison)	11 August
-	Berries ripe for harvest	15 September

Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has very high sugar and medium acidity content.








Tedotsminda 03

Passport information	
Number in collection	GEO038-W2014-035
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Tedotsminda is a village in Gori district of Inner Kartli, where the plant was descovered.

Historical facts and distribution

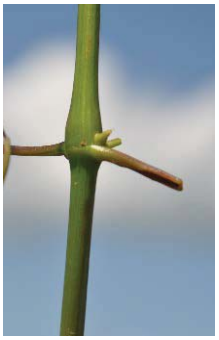
The plant was detected in the right bank of river Liakhvi, in 2012. The plant grew in North-directed slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	3	low
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	4	copper-reddish
OIV053	Density of prostrate hairs between main veins on lower side of blade	3	low
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	1	absent or very weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
OIV087	Density of erect hairs on main veins on lower side of blade	5	medium
OIV094	Depth of upper lateral sinuses	3	shallow

Tedotsminda 03

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-



Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	19 April
-	Beginning of flowering	1 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available

Tedotsminda 04

Passport information	
Number in collection	GEO038-W-2014-025
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Tedotsminda is a village in Gori district of Inner Kartli, where the plant was descovered.

Historical facts and distribution

The plant was detected in the right bank of river Liakhvi, in 2012. The plant grew in North-directed slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	7	high
Shoot			
OIV006	Attitude (before tying)	5	horizontal
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	5	medium
Young (4th) leaf			
OIV051	Color of upper side of blade	1	green
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	1	absent
OIV072	Goffering of blade	5	medium
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	5	mixture between both sides straight (note 2) and both sides convex (note 3)
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	9	present
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	5	medium

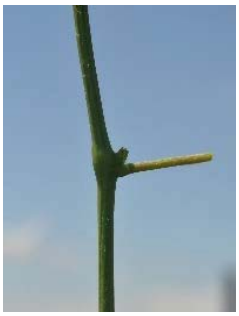
Tedotsminda 04

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1	very short
OIV203	Width	1	very narrow
OIV204	Density	1	very loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	1	very short
OIV221	Width	1	very narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	5	medium
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	7	high
OIV506	Total acidity of must	5	medium
OIV508	Must specific pH	5	medium

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	1816
Total polyphenols	2502

Phenotyping trial		
OIV301	Time of bud burst	18 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	13 August
-	Berries ripe for harvest	15 September









Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has high sugar and medium acidity content

Tedotsminda 16

Passport information	
Number in collection	GEO038-W2014-023
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Tedotsminda is a village in Gori district of Inner Kartli, where the plant was descovered.

Historical facts and distribution

The plant was detected in the right bank of river Liakhvi, in 2012. The plant grew in North-directed slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	3	low
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	3	red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	-	-
Young (4th) leaf			
OIV051	Color of upper side of blade	4	copper-reddish
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	1	absent or very weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	1	absent or very weak
OIV076	Shape of teeth	5	mixture between both sides straight (note 2) and both sides convex (note 3)
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	1	U-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	9	present
OIV084	Density of prostrate hairs between main veins on lower side of blade	1	none or very low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	5	medium

Tedotsminda 16

Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	5	medium
OIV203	Width	3	narrow
OIV204	Density	5	medium
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	2	conical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	3	short
OIV221	Width	3	narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	5	medium
OIV235	Firmness of flesh	2	slightly firm
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	7	high
OIV508	Must specific pH	5	medium

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mMg / kg in grapes
Total anthocyanins	1439
Total polyphenols	2502

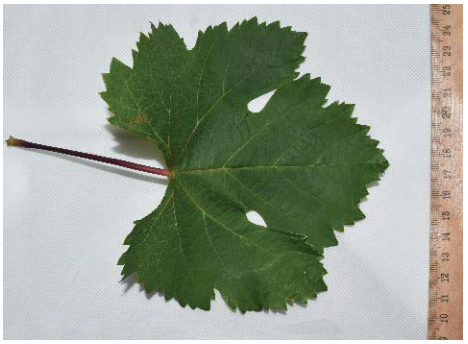
Phenotyping trial		
OIV301	Time of bud burst	20 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	11 August
-	Berries ripe for harvest	7 September

Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has very high sugar and acidity content.









Tedotsminda 22

Passport information	
Number in collection	GEO038-W2014-029
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Tedotsminda is a village in Gori district of Inner Kartli, where the plant was descovered.

Historical facts and distribution

The plant was detected in the right bank of river Liakhvi, in 2012. The plant grew in North-directed slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
ყლორტი			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	green
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	1	absent
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	1	absent or very weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	3	shallow

Tedotsminda 22

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	3	low

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

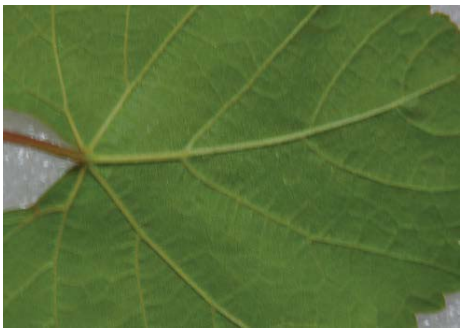
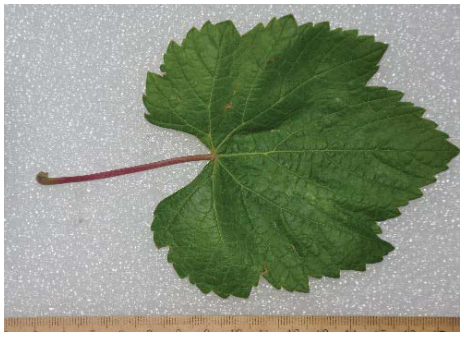
Phenotyping trial		
OIV301	Time of bud burst	25 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

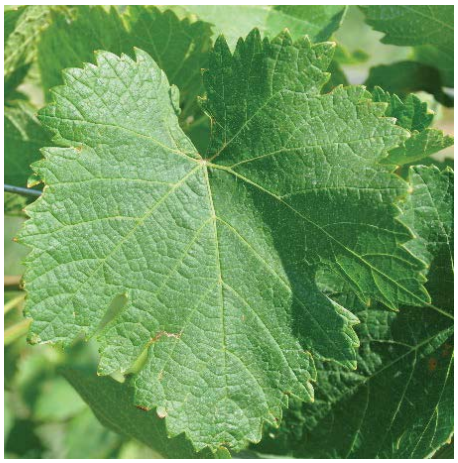
No grapes are available.






Tedotsminda 23



Passport information	
Number in collection	GEO038-W2014-027
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Tedotsminda is a village in Gori district of Inner Kartli, where the plant was descovered.

Historical facts and distribution

The plant was detected in the right bank of river Liakhvi, in 2012. The plant grew in North-directed slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	3	low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	5	horizontal
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	5	medium
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	3	five
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	1	absent
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	2	on one side
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	5	medium

Tedotsminda 23

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	5	medium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	20 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.







Tedotsminda 25

Passport information	
Number in collection	GEO038-W2014-022
Color of berry	Black
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

Tedotsminda is a village in Gori district of Inner Kartli, where the plant was descovered.

Historical facts and distribution

The plant was detected in the right bank of river Liakhvi, in 2012. The plant grew in North-directed slope. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	5	horizontal
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV0155	Fertility of basal buds (buds 1-3)	5	medium
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	3	pentagonal
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	3	up to the 1st bifurcation
OIV072	Goffering of blade	1	absent or very weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	1	absent or very weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	1	none or very low
OIV094	Depth of upper lateral sinuses	3	shallow

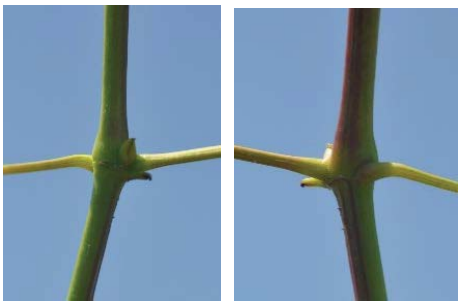
Tedotsminda 25

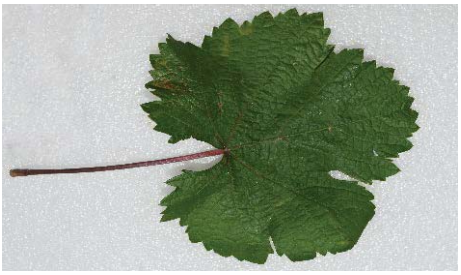
Flower			
OIV151	Sexual organs	4	reflexed stamens and fully developed gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	1	very short
OIV203	Width	1	very narrow
OIV204	Density	1	very loose
OIV206	Length of peduncle of primary bunch	3	short
OIV208	Shape	1	cylindrical
OIV209	Number of wings of the primary bunch	2	1 – 2 wings
Berry			
OIV220	Length	1	very short
OIV221	Width	1	very narrow
OIV223	Shape	2	globose
OIV225	Color of skin	6	blue black
OIV231	Intensity of flesh anthocyanin coloration	1	none or very weak
OIV235	Firmness of flesh	1	soft
OIV236	Particular flavor	1	none
OIV241	Formation of seeds	3	complete
Elements of productivity			
OIV502	Single bunch weight	1	very low
OIV503	Single berry weight	1	very low
OIV504	Harvest m^2	1	very low
Characteristics of grape juice			
OIV505	Sugar content of must	9	very high
OIV506	Total acidity of must	5	medium
OIV508	Must specific pH	5	medium

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to *Plasmopara* (leaf disc test)	3	low

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	1709
Total polyphenols	2812

Phenotyping trial		
OIV301	Time of bud burst	16 April
-	Beginning of flowering	2 June
OIV303	Time of beginning of berry ripening (veraison)	8 August
-	Berries ripe for harvest	6 September

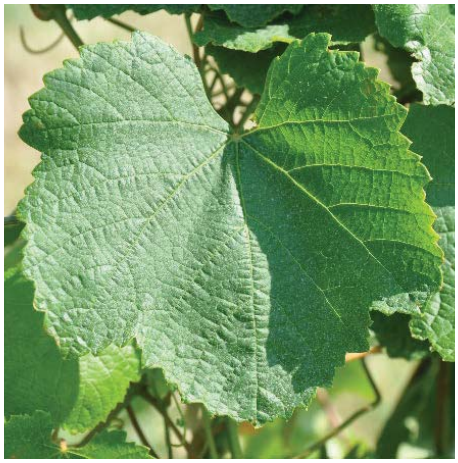




Characterization of wine and grapes

The bunch is small, sparse, with small black berries. The must has very high sugar content and medium acidity.

Tushis Tbebi 01



Passport information	
Number in collection	GEO038-W2014-019
Color of berry	-
Taxonomy	*Vitis vinifera* subs *sylvestris* (C.C.Gmel.) Hegi
Country	Georgia

Synonyms, meaning of name

'Tushis Tbebi' is a name of the locality in Sagarejo district of Kakheti, where the plant was descovered.

Historical facts and distribution

The plant was detected in the left bank of Iori river, in 2006. The plant grew in lowland riparian forest. Recently it is preserved in Jighaura collection.

Basic ampelographic characters

Young shoot			
OIV001	Opening of the shoot tip	5	fully open
OIV003	Intensity of anthocyanin coloration on prostrate hairs of the shoot tip	1	none or very low
OIV004	Density of prostrate hairs on the shoot tip	5	medium
Shoot			
OIV006	Attitude (before tying)	3	semi-erect
OIV007	Color of the dorsal side of internodes	2	green and red
OIV008	Color of the ventral side of internodes	1	green
OIV016	Number of consecutive tendrils	1	2 or less
OIV155	Fertility of basal buds (buds 1-3)	9	very high
Young (4th) leaf			
OIV051	Color of upper side of blade	3	bronze
OIV053	Density of prostrate hairs between main veins on lower side of blade	7	high
Mature leaf			
OIV067	Shape of blade	2	wedge-shaped
OIV068	Number of lobes	2	three
OIV070	Area of anthocyanin coloration of main veins on upper side of blade	1	absent
OIV072	Goffering of blade	3	weak
OIV074	Profile of blade in cross section	3	involute
OIV075	Blistering of upper side of blade	3	weak
OIV076	Shape of teeth	3	both sides convex
OIV079	Degree of opening / overlapping of petiole sinus	3	half open
OIV080	Shape of base of petiole sinus	3	V-shaped
OIV081-1	Teeth in the petiole sinus	1	absent
OIV081-2	Petiole sinus base limited by vein	1	not limited
OIV083-2	Teeth in the upper lateral sinuses	1	absent
OIV084	Density of prostrate hairs between main veins on lower side of blade	3	low
OIV087	Density of erect hairs on main veins on lower side of blade	3	low
OIV094	Depth of upper lateral sinuses	1	absent or very shallow

Tushis Tbebi 01

Flower			
OIV151	Sexual organs	1	fully developed stamens and no gynoecium
Woody shoot			
OIV103	Main color	2	brownish
Bunch			
OIV202	Length (peduncle excluded)	-	-
OIV203	Width	-	-
OIV204	Density	-	-
OIV206	Length of peduncle of primary bunch	-	-
OIV208	Shape	-	-
OIV209	Number of wings of the primary bunch	-	-
Berry			
OIV220	Length	-	-
OIV221	Width	-	-
OIV223	Shape	-	-
OIV225	Color of skin	-	-
OIV231	Intensity of flesh anthocyanin coloration	-	-
OIV235	Firmness of flesh	-	-
OIV236	Particular flavor	-	-
OIV241	Formation of seeds	-	-
Elements of productivity			
OIV502	Single bunch weight	-	-
OIV503	Single berry weight	-	-
OIV504	Harvest m^2	-	-
Characteristics of grape juice			
OIV505	Sugar content of must	-	-
OIV506	Total acidity of must	-	-
OIV508	Must specific pH	-	-

Degree of resistance to Plasmopara			
OIV452-1	Leaf: degree of resistance to Plasmopara (leaf disc test)	5	me dium

Biochemical characteristics	mg / kg in grapes
Total anthocyanins	-
Total polyphenols	-

Phenotyping trial		
OIV301	Time of bud burst	21 April
-	Beginning of flowering	1 June
OIV303	Time of beginning of berry ripening (veraison)	-
-	Berries ripe for harvest	-

Characterization of wine and grapes

No grapes are available.

Chapter 11

Dissemination

Una aproximación a los orígenes de la viticultura, del vino y a la figura del científico Nikolái Vavílov

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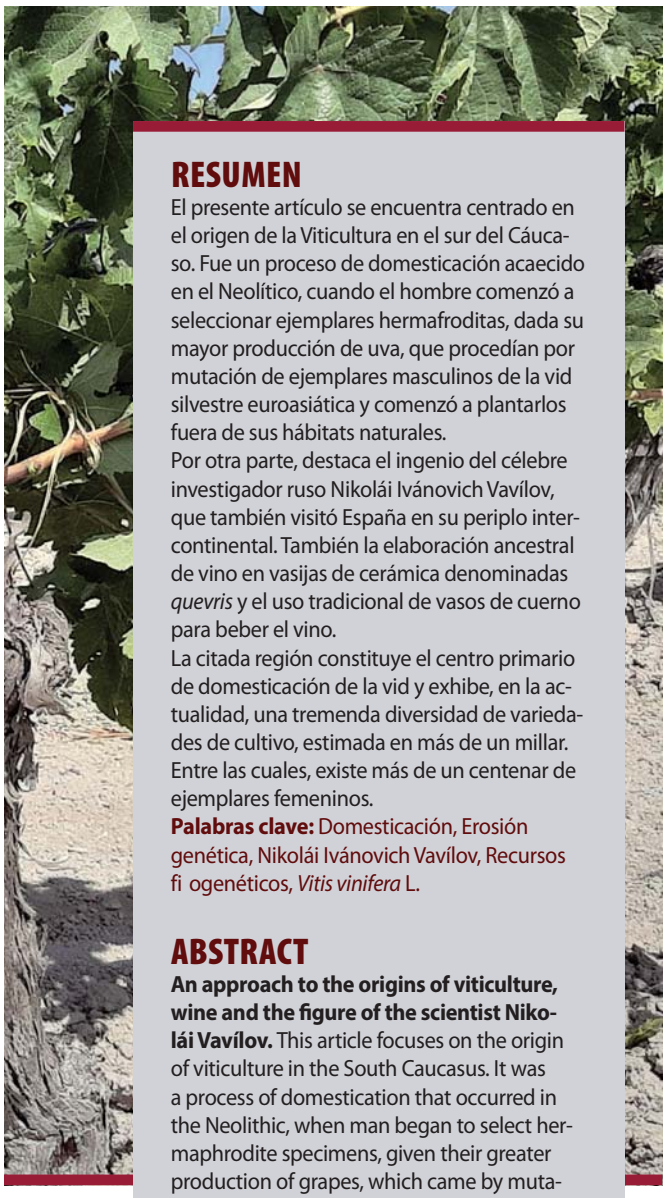

RESUMEN

El presente artículo se encuentra centrado en el origen de la Viticultura en el sur del Cáucaso. Fue un proceso de domesticación acaecido en el Neolítico, cuando el hombre comenzó a seleccionar ejemplares hermafroditas, dada su mayor producción de uva, que procedían por mutación de ejemplares masculinos de la vid silvestre euroasiática y comenzó a plantarlos fuera de sus hábitats naturales.

Por otra parte, destaca el ingenio del célebre investigador ruso Nikolái Ivánovich Vavílov, que también visitó España en su periplo intercontinental. También la elaboración ancestral de vino en vasijas de cerámica denominadas *quevris* y el uso tradicional de vasos de cuerno para beber el vino.

La citada región constituye el centro primario de domesticación de la vid y exhibe, en la actualidad, una tremenda diversidad de variedades de cultivo, estimada en más de un millar. Entre las cuales, existe más de un centenar de ejemplares femeninos.

Palabras clave: Domesticación, Erosión genética, Nikolái Ivánovich Vavílov, Recursos fi ogenéticos, *Vitis vinifera* L.

ABSTRACT

An approach to the origins of viticulture, wine and the figure of the scientist Nikolái Vavílov. This article focuses on the origin of viticulture in the South Caucasus. It was a process of domestication that occurred in the Neolithic, when man began to select hermaphrodite specimens, given their greater production of grapes, which came by mutation of male specimens of the Eurasian wild grapevine and began to plant them outside their natural habitats.

On the other hand, the ingenuity of the famous Russian researcher Nikolái Ivánovich Vavílov, who also visited Spain on his intercontinental journey, is stood out. Also the ancestral elaboration of wine in ceramic pots, called *quevris,* and the traditional use of drinking horns for wine.

The aforementioned region constitutes the primary center of domestication of the grapevine and exhibits, at present, a tremendous diversity of crop varieties, estimated at more than a thousand. Among which, there are more than a hundred of female specimens.

Key words: Domestication, Genetic erosion, Nikolái Ivánovich Vavílov, Plant genetic resources, *Vitis vinifera* L

El presente artículo trata de reflejar la importancia del Triángulo de la Uva Fértil de Vavílov, como el centro primario de domesticación de la vid euroasiática. Este alberga, por lo tanto, su mayor biodiversidad en la importante cantidad de poblaciones de vid silvestre como en la elevada cantidad de variedades de cultivo, tanto hermafroditas como femeninas. Comenta la curiosidad de que el relato bíblico indica que el arca de Noé encalló en el Monte Ararat, situado en la zona central del citado polígono geográfico.

Por otra parte, destaca el ingenio del célebre investigador ruso Nikolái Ivánovich Vavílov. También, recoge pruebas arqueológicas de los inicios de la Viticultura y producción de vino desde el Neolítico en Transcaucasia. Resalta, además, que la producción enológica en las tinajas georgianas, llamadas *quevri*, constituye, en la actualidad, un Patrimonio Cultural Inmaterial de la Humanidad desde 2014 (UNESCO, 2021). También ofrece información sobre los destilados armenios y destaca la importancia de las parras silvestres y variedades de cultivo en la región como un importante recurso fitogenético.

Esta región, a caballo entre Europa y Asia, por donde discurría un tramo de la Ruta de la Seda y que formó parte de la URSS, ha tenido, por su posición estratégica, varios conflictos bélicos. Entre los más recientes del presente siglo se encuentran la ocupación de Ossetia del Sur y Adjasia, puerta del mar Negro, por las tropas rusas, que continúa en la actualidad. Y, también, el enfrentamiento entre Armenia y Azerbaiyán por el territorio de Nagorno Karabaj.

El refugio de la vid silvestre durante la última glaciación en Transcaucasia

La cordillera del Cáucaso queda dividida en dos sistemas montañosos: el Gran Cáucaso, al norte, y el Cáucaso menor, que se extienden desde el mar Negro hasta el Caspio. Entre ambos discurre la llamada Depresión Transcaucásica. El pico más elevado es el Monte Elbrús, con algo más de 5.600 m de altitud, también existen otros cuatro más que superan los 5.000 m.

Los primeros fósiles de vitáceas del sur del Cáucaso se encuentran en estratos mesozoicos datables entre los 130 y 200 millones de años. Huellas foliares de la especie *Vitis zaisanica* Balk. aparecen en los alrededores de la localidad de Koturvan, en la provincia armenia de Vayots Dzor, en rocas sedimentarias del Plioceno Inferior, con una antigüedad que oscila entre los 4–5 millones de años (Gokhtuni, 1963; Harutyunyan *et al*., 2005).

Las citadas cordilleras ejercieron una protección sobre determinados ecosistemas, principalmente bosques de ribera, llanuras de inundación y faldas de colinas de baja altitud, frente a los hielos y fríos vientos de componente norte durante la última etapa glaciar del Cuaternario (Würm) (Huglin, 1986). En ellos, se refugiaron varios antecesores de especies productoras de frutos para el consumo humano, como son almendros, perales, melocotoneros, granados, entre otros. También, las poblaciones de parras silvestres euroasiáticas (*Figura 1*).

Varios taxones relictos en las regiones cálidas y húmedas del sur del Cáucaso, entre los que figura *Vitis vinifera* subsp. *sylvestris* (C.C.Gmel.) Hegi, ocupan nichos en bosques pantanosos que podrían representar *entornos primitivos* de especies que en la actualidad también son elementos de la vegetación mesomediterránea y de los bordes y setos de bosques termófilos en Europa Central (Denk *et al*., 2001).

Otros relictos terciarios, que suelen coincidir en los mismos hábitats de la vid silvestre en la región, son *Crataegus monogyna* subsp. *curvisepala, Morus alba, Smilax excelsa, Pterocarya fraxinifolia,* y *Quercus hartwissiana* (Denk *et al*., 2001).

Figura 1. Parras silvestres en el bosque de ribera del río Guruchai (Azerbaiyán).

En la actualidad se conservan, a nivel mundial, unas 70 especies silvestres, del género *Vitis* L., que contienen 38 cromosomas. La mayor parte de ellas se encuentran en Estados Unidos y China (Martínez de Toda, 1991).

Las poblaciones de vid silvestre euroasiática se distribuyen entre la Península Ibérica y el Macizo del Hindhu Kush. Su franja latitudinal se encuentra limitada por los paralelos 30–31 (río Ourika, situado a los pies de la Cordillera del Atlas, Marruecos) y 49–50 (en el Stadt Park de Lwfdishaffen, junto al río Rin, Alemania). Desde el punto de vista botánico, pertenecen al taxon *Vitis vinifera* L. subespecie *sylvestris* (Gmelin) Hegi. Se trata de una subespecie dioica; es

Figura 2. Síntomas de erinosis en el envés de una hoja.

decir contienen ejemplares con flores masculinas y otros con flores femeninas. Mientras que las vides cultivadas, seleccionadas por el hombre, como se indicará posteriormente, a partir de este parental silvestre, se incluyen dentro de la subespecie *Vitis vinifera* L. subespecie *sativa* (DC.) Hegi. Sus ejemplares son mayoritariamente hermafroditas. Y se utilizan como uva de mesa, para pasificación, preparación de mostos, vinificación y obtención de cosméticos.

Ecología de la vid silvestre en la región (hábitats y estado sanitario)

La vid silvestre (*Vitis vinifera* subsp. *sylvestris* (Gmelin) Hegi es una trepadora leñosa portadora de zarcillos que habita en los bosques y matorrales a lo largo de las riberas de los ríos y los cauces de los barrancos, desde Europa occidental hasta Asia central. En la zona del sur del Cáucaso se encuentra particularmente disperso a lo largo de cursos de agua caudales bajos. Como liana, *Vitis vinifera* subsp. *sylvestris*, aunque leñosa, no puede permanecer de pie hasta una altura apreciable. Para trepar, las enredaderas deben ubicarse y de alguna manera agarrarse, apoyarse o engancharse en soportes adecuados (Putz y Mooney, 1991).

En la actualidad, los estudios sobre lianas o enredaderas y las especies hospedadoras que brindan su soporte mecánico son escasos, aunque las enredaderas pueden exhibir una especificidad de hospedante basada en la identidad, tamaño o forma de la especie arbórea (Ladwig y Meiners, 2015; Muñoz *et al*., 2003).

Una prospección de hábitats y especies hospedadoras que sirven de apoyo mecánico para la vid silvestre, trepadora, que se llevó a cabo en 13 poblaciones naturales situadas a lo largo de bosques ribereños, llanuras aluviales y coluviones en Georgia (distritos de Marneuli, Mtskheta y Gori, área protegida de Gardabani y reserva de Lagodekhi), Armenia (regiones de Akhtala y Tavoush) y Azerbaiyán (Región de Quba) permitió determinar las especies más relevantes (Ocete *et al*., 2018). Los resultados del estudio muestran cuatro grandes grupos de especies asociados a características topográficas y áreas biogeográficas. Los árboles de *Punica granatum* son el soporte mecánico más común para la vid silvestre en el sur del Cáucaso (*Figura 1*).

El número de plagas y enfermedades que afectan a estas vitáceas silvestres autóctonas es bastante reducido. El grado de infestación e infección varía de un ejemplar a otro, dentro cada población. Dicha diferencia de grados de sensibilidad mostrados por distintos ejemplares de una misma población, que, a veces, se encuentran en contacto físico, constituye un exponente de la diversidad genética de sus componentes, ya que la reproducción es generalmente por semilla (es decir, sexual) (Maghradze *et al*., 2009).

En lo referente a artrópodos, la especie más común es *Colomerus vitis* (Pagenstecher) (Acari, Eryophidae). De las tres razas existentes so-

Figura 3. Síntomas de mildiu sobre hojas.

bre el viñedo, únicamente se ha encontrado la causante de erineos (erineum strain) en las hojas (falsas agallas), que constituyen los nidos del ácaro. Al picar en el limbo foliar, forman unas protuberancias en el haz, cuya parte correspondiente al envés se encuentra recubierta de tricomas, pilosidades desarrolladas por las células epidérmicas al inyectar su saliva (Ocete *et al*., 2007) (*Figura 2*). Una presencia mucho más baja es la de otro eriófido, *Calepitrimerus vitis* (Nalepa) (Acari, Eriophydae), que provoca la aparición de brotes arrugados con un desarrollo ralentizado. Posteriormente, aparecen puntos decolorados en la hoja, por la succión de savia del ácaro (Maghradze *et al*., 2010 y 2015).

Los principales problemas sanitarios son los causados por los hongos patógenos de origen norteamericano, importados de Estados Unidos durante el s. XIX o comienzos del XX, el oídio, *Erysiphe necator* (Burr.), y el mildiu, *Plasmopara viticola* (Berk. y Curt.) Berl. y de Toni. Desde su llegada en la segunda mitad del s. XIX, con las especies de vides de dicho origen utilizadas como portainjertos, probablemente han reducido bastante el número de parras que se conservan en cada población (Ocete *et al*., 2012; Maghradze *et al*., 2012). Por ello, seguramente, ya no se encuentran aquellos monumentales ejemplares de gran antigüedad que, según Pallas (1799–1801), científico alemán que trabajó para la emperatriz Catalina La Grande de Rusia, llegaban a tener "*dimensiones de tronco similares a mástiles de barco*" (*Figuras 4* y *5*).

No se han apreciado en ningún ejemplar de Armenia, Azerbaiyán y Georgia síntomas causados por el virus del entrenudo corto (Grape fanleaf virus, GFLV).

A nivel de los pelos absorbentes de la raíz, hay que destacar que en ningún caso se han detectado síntomas causados por la filoxera, *Daktulosphaira vitifoliae* (Fitch) (Hemiptera, Phylloxeridae), debido a las condiciones edáficas, como

Figura 4. Retrato de Peter Simon Pallas.

Figura 5. Pelos absorbentes carentes de síntomas de filoxera y de micelios de podredumbre radicular.

son el encharcamiento y/o la textura arenosa del suelo. No obstante, la vid silvestre es sensible al pulgón norteamericano, ya que, si se ponen plántulas en maceta y se recubre la tierra con hojas de portainjertos infestadas agallas del insecto, se producen síntomas de infestación, al cabo de unos 8–9 meses en distintas muestras poblacionales europeas (Ocete *et al*., 2011).

Otro hecho llamativo es la ausencia de micelios de hongos causantes de podredumbres de la raíz, como son *Armillaria mellea* (Vahl.) y *Rosellinia necatrix* (Hartig), aunque varios de sus tutores sí tienen síntomas de este problema sanitario (*Figura 5*).

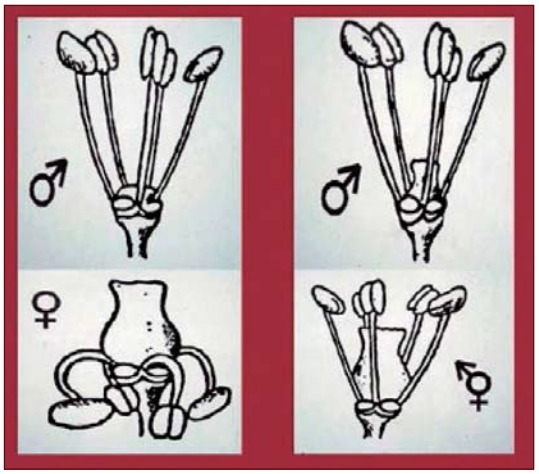

Figura 6. Tipos de flores: masculinas (Tipo I y II), femenina y hermafrodita. Julius Kühn Institut. Siebeldingen (Alemania).

Principales características ampelográficas de las parras silvestres

Las parras masculinas normalmente presentan hojas más pequeñas y lobuladas que las femeninas. Las inflorescencias, por el contrario, son de mayor tamaño que las de las femeninos. Tienen que producir una gran cantidad de polen porque la fecundación es fundamentalmente anemógama. Exhalan un olor característico, motivo por el que en algunas producciones locales de Azerbaiyán se solían macerar en las vasijas con vinos blancos del año anterior, para conferirles un aroma más afrutado, una práctica que también se hacía en España (Laguna, 1570).

La mayor parte de los ejemplares presentan flores, con 5 estambres erguidos y carecen de gineceo (Tipo I). No obstante, en una proporción muy inferior, pueden verse parras masculinas con un gineceo poco desarrollado (Tipo II).

Las flores femeninas muestran un gineceo bien patente y 5 estambres reflejos (curvados hacia abajo) (*Figura 6*).

El polen masculino de los estambres es muy abundante y tricorporado; es decir, con orificios para la salida del tubo polínico por donde se evacúan los gametos para la fecundación de las flores del sexo opuesto. Por el contrario, el polen femenino es escaso y carece de dichos orificios, por lo que no pueden autofecundarse. Debido a ello, la polinización es cruzada.

La inmensa mayoría de las plantas femeninas producen racimos de uvas tintas con tamaño inferior a 1 cm de diámetro, con la pulpa no coloreada. Contienen entre 1 y 4 semillas. Estas son más redondeadas, con un pico mucho menos patente que el correspondiente a las procedentes de ejemplares de cultivo. Por este motivo, en los yacimientos arqueológicos se puede averiguar si la gente de una determinada época consumía vid silvestre o cultivada (*Figura 6*).

El perfil analítico de los vinos de vid silvestre femenina

Los parámetros analíticos de los vinos silvestres obtenidos en la región, siempre con bayas tintas, tras un despalillado manual, distan mucho de los correspondientes a los vinos obtenidos de cultivares tintos en cualquier zona del mundo. Los datos que se muestran proceden de las microvinificaciones llevadas a cabo por Maghradze *et al.* (2020), que fueron analizadas por el equipo de la Estación Enológica de Haro (La Rioja).

El porcentaje de etanol de las muestras varía entre 5,04 y 10,15 g/L. La intensidad del color entre 2,59 y 20,76. Según los datos expuestos, en general, el porcentaje de etanol y la intensidad del color son inferiores a los valores registrados en microvinificaciones de varias regiones españolas, como Andalucía, La Rioja, Castilla y León y Navarra (Ocete *et al*., 2011a yb). Es necesario destacar que las poblaciones del sur del Cáucaso pertenecen a clorotipos diferentes al de las parras silvestres españolas (Arroyo–García *et al*., 2006; De Andrés, *et al*., 2012), como se recoge al final de este artículo.

Todas las muestras presentan diferentes concentraciones residuales de azúcares reductores, probablemente debido al alto índice de polifenoles totales y la alta acidez, que alteran la acción de las levaduras autóctonas para apurar la fermentación alcohólica.

Se han determinado algunas muestras con valores de ácido acético, superiores a 0,5 g/L. Estos también son habituales en vinos de cultivares hermafroditas que han sufrido una fermentación maloláctica deficiente. Únicamente provocan un aumento de la acidez total de aproximadamente 1 g/L. Por ello, los elevados valores de acidez total encontrados en algunas microvinificaciones se deben a la concentración de ácido málico no transformado en láctico.

Estos vinos podrían asemejarse a los correspondientes a las antiguas producciones de esta bebida psicotrópica, elaboradas en esta zona antes del proceso de domesticación de la vid.

Breve reseña sobre Vavílov y su visita a España

Hay que destacar que pocos científicos habrán sido tan intelectualmente ambiciosos como Nikolái Ivánovich Vavílov (1887–1943). Al frente del Instituto de Botánica Aplicada (actualmente VIR) de San Petersburgo. Su objetivo fue aumentar la producción agrícola mediante mejora genética para eliminar, o reducir, las hambrunas que periódicamente asolaban la Unión Soviética. Para ello planteó la necesidad de conocer todas las plantas cultivadas del globo, su genética, su variabilidad, su fisiología... con la mayor amplitud y profundidad que fuera posible. Necesitaba desarrollar mucha ciencia básica para aplicarla a la resolución de problemas.

La mente de Nikolái era sistemática, de modo que la exploración del mundo, es decir, la búsqueda de los «*centros de origen de cada cultivo*» (un concepto creado por él mismo), también debía serlo. Suponía que el centro originario de cada cultivo contenía la mayor diversidad y, por tanto, su máxima variabilidad genética, tanto de ejemplares silvestres como cultivados. En el caso de la vid, descubrió que su centro originario se encontraba situado entre los mares Negro y Caspio, cubriendo la zona del sur del Cáucaso, y limitado por los montes Taurus (Anatolia, Turquía) y Zagros (Irán) (Vavílov, 1926). Esta zona geográfica tiene forma triangular, como se aprecia en la *Figura 7*. Por tal motivo, fue bautizada

Figura 7. Triángulo de la Uva Fértil de Vavílov.

por otro investigador soviético, Negrul (1938), como Triángulo de la Uva fértil o Triángulo de Vavílov. Desde aquí, paulatinamente, se fue irradiando el cultivo de esta vitácea hacia Mesopotamia y Oriente Medio. El flujo de cultivares tuvo lugar desde las áreas orientales del Mediterráneo hasta el Oeste del mismo (Marsal, 2015). Su introducción en España se debe a su importación por fenicios y griegos.

Vavílov emprendió personalmente numerosas expediciones y organizó muchas otras más, cuyo resultado, entre otras cosas, fue la creación del mayor banco de germoplasma de plantas cultivadas del mundo. Al final inesperado de su vida, quiso narrar sus propios viajes en una obra en dos volúmenes que titularía *Cinco continentes*.

Dedicó el año de 1926 a estudiar la agricultura de los países ribereños del Mediterráneo. El proyecto (además del estudio de Abisinia) culminó con la visita a la Península Ibérica, a la que llegó en junio de 1927. Desde Barcelona se trasladó directamente a Madrid, donde le recibieron el director del Museo de Ciencias Naturales, el entomólogo Ignacio Bolívar, y el botánico Luis Crespí, que le acompañó y le sirvió de ayuda durante su estancia. Era la época de la dictadura de Primo de Rivera. Aunque Vavílov tenía visado para una estancia de un mes, que prorrogó con la ayuda de Crespí por otros dos meses más, la vigilancia policial, al provenir de territorio comunista, fue constante durante el tiempo que permaneció aquí, con alguna anécdota chusca que él mismo relata con humor.

El relato de su viaje por España es uno de los más completos que aparecen en *Cinco continentes*. Como es sabido, la obra hubo de quedar inconclusa y fragmentaria, tras la detención de Vavílov en 1940 y su posterior fallecimiento en prisión en 1943, en condiciones penosas. Entonces Stalin presidía la URSS y, como todos los dictadores, tenía *mala uva*, a pesar de haber nacido en Georgia, cuna de la viticultura, concretamente en la ciudad de Gori.

Muchos de los países y los temas que nos promete el índice general (que sí se ha conservado y ocupa un buen número de páginas) se quedaron sin narración, o bien esta se perdió irremediablemente (como ocurre singularmente, y por desgracia, con Portugal, que visitó en este mismo periodo).

Con Madrid como centro general de operaciones, Vavílov visitó prácticamente toda la península: el centro y las tierras manchegas, el Levante, Andalucía, Portugal, las extensiones cerealistas de Castilla la Vieja y el norte, Galicia, Asturias y Navarra. Como era su proceder en todas sus expediciones, trató de visitar todas las regiones agrícolas ibéricas, estudiando sus cultivos, recogiendo muestras en cantidades copiosas que enviaba regularmente a su instituto de San Petersburgo y, en definitiva, tratando de entender las líneas generales de la agricultura local, sus diversas derivaciones e influencias históricas, lo que él llamaba la 'filosofía' de cada país.

Lo que vio le impactó mucho. «*España resultó un país realmente interesante para entender la agricultura europea*», escribe. En aquella época el mundo rural era absolutamente diferente del actual y las particularidades locales mucho más marcadas que ahora, que casi no existen. A Vavílov le llamaron la atención algunos cultivos endémicos (o casi), como las afreitas o avenas negras de Galicia (avenas diploides), singulares especies de leguminosas entonces muy cultivadas como la algarroba (*Vicia articulata*) o el alberjón (*Vicia narbonensis*), el tojo (*Ulex europaeus*) cultivado como planta forrajera en Galicia y la espelta asturiana, entre otras. Señaló también el interés que tenían las variedades de leguminosas de semilla grande que había encontrado, sobre todo garbanzos, habas y almortas, el variado surtido de trigos (hoy prácticamente desaparecido), las variedades de hortalizas de tamaño excepcional y una muy interesante diversidad de árboles frutales, sobre todo del sur de la península, que «*merece atención para su uso en las secas regiones subtropicales soviéticas*» (Vavílov, 2015).

A mediados de agosto, después de visitar Navarra y constatar la gran cantidad de variedades de escaña (trigos tetraploides) que allí se cultivaban, cruzó la frontera por Irún para dirigirse a Alemania, en donde le esperaba el 5º Congreso Internacional de Genética, que se reuniría en Berlín del 11 al 18 de septiembre.

Figura 8. Vavílov en su despacho de San Petesburgo. Foto cedida por VIR, San Petersburgo (Rusia).

Leyendo su relato, no cabe duda de que a Vavílov le emocionaron los descubrimientos que hizo en este viaje. Y no fueron únicamente los cultivos que pudo encontrar, también los agricultores y otras personas que le ayudaron en sus pesquisas, pues él era un hombre atento a todo. El lector actual percibirá seguramente, y lamentará, la pérdida de diversidad agrícola acaecida en los cien años que nos separan de aquella época, pero también participará de la emoción de este hombre que quiso «*estudiar el planeta entero*» (*Figura 8*).

Las tradiciones de Noé y Santa Nino

Dentro del citado triángulo geográfico, se encuentra el Monte Ararat, verdadero símbolo identificativo de Armenia. Este fue el lugar donde según la tradición judeo–cristiana, inspirada en el Libro del Génesis, encalló el arca de Noé. Personaje que plantó la primera viña y con su vino se embriagó. Esta zona geográfica pertenecía a Armenia pero fue ocupada durante la Primera Guerra Mundial por las fuerzas turcas, responsables del llamado *genocidio armenio*, que se consumó en 1920.

El citado libro integrante de la Biblia señala textualmente: «*Ante la corrupción de la humanidad, Dios decidió su exterminio* (Génesis [Gn]

Figura 9. Viñedo armenio con el Monte Ararat al fondo.

6,5–12). *Pero a Noé, el varón más justo y cabal de su tiempo, decidió salvarlo en un arca y establecer una alianza gratuita con él* (Gn 6,13–22). *Tenía Noé 600 años al embarcarse con su familia y las parejas de animales. Diluvió durante cuarenta días con sus noches. Las aguas cubrieron hasta los montes y la inundación duró ciento cincuenta días* (Gn 7,1–24). Cuando *Dios quiso, cesó la lluvia y el viento secó la tierra* (Gn 8,1–22). *El arca quedó varada sobre los* ***montes de Ararat*** (Gn 8,4)».

En el Antiguo Testamento, Ararat no designa concretamente el monte, sino únicamente la región de Armenia, junto al curso medio del río Araxes, contigua a la parte septentrional de Asiria. Es el imperio de Urarfu, de las inscripciones cuneiformes, que floreció en los siglos IX–VII a. C. Solo posteriormente se dio el nombre de la región a una sola montaña (Haag, 1951; De Ausejo, 1978).

No sabemos quién escribió esos relatos bíblicos, pero sí está claro que conocía la gran diversidad de vides, tanto silvestres como cultivadas, que poblaban y aún se conservan en la región. De hecho, dentro del citado Triángulo de la Uva Fértil de Vavílov, que alberga la máxima diversidad de la vid, el Monte Ararat se encuentra en la zona central del mismo (*Figuras 9, 10 y 11*).

Según la tradición ortodoxa, Santa Nino introdujo el cristianismo en Georgia en el s. IV. Era una joven que procedía de La Capadocia (actual Turquía). Venía acompañada de sus padres y portaba una cruz formada por dos fragmentos de tronco de vid fijados entre sí con sus propios cabellos. En la catedral de Tbilisi puede contemplarse la posible reliquia. De hecho, su imagen se encuentra en la práctica totalidad de las iglesias de país (*Figura 12*).

Primeras pruebas de domesticación de la vid

Su domesticación y cultivo comenzó durante el Neolítico. Prueba de ello ha sido el hallazgo de semillas con morfología claramente perteneciente a cepas cultivadas, menos rechonchas y con un pico más alargado, en Shulaveri Gora (Georgia). Concretamente en un nivel arqueológico datable hacia el 6.000 a.C. Estas pepitas fueron descubiertas por investigadores soviéticos en las campañas de excavación acaecidas durante la década de 1960 (Mc Govern, 2003 y 2004; Chilashvili, 2004), en las que las catas palinológicas revelaron, también, la presencia de abundantes granos de polen tricorporado (*Figuras 13, 14* y *15*).

Figura 10. Decoración del interior de la Bodega Noy (Ereván), en la que se representa la salida del Арса.

Figura 11. Cubeta de la bodega anterior en la que se representa el Arca sobre el Monte Ararat. La inscripción en ruso dice : *Noé, señor supremo.*

Lógicamente, en aquella época los grupos humanos desconocían la sexualidad de las parras silvestres, pero seleccionaron las hermafroditas, aparecidas por mutación de algunos ejemplares masculinos, ya que al poseer autofecundación tenían un mayor nivel de cosecha.

Esa zona arqueológica constituye el origen de la llamada "*Cultura Shulaveri Shomutepe*" del período Neolítico, que se extiende hacia el oeste de Azerbaiyán y el norte de Armenia. Y constituye el primer yacimiento en el que los arqueólogos encontraron restos cerámicos de varias vasijas para vinificación, conocidas como *proto–quevris*. Algunas con cerca de 300 litros de capacidad y una antigüedad compatible con la de las primeras semillas cultivadas.

Figura 12. Icono de Sta. Nino portando la cruz hecha con sarmientos.

Los análisis químicos de compuestos orgánicos antiguos absorbidos en la cerámica de yacimientos de Georgia, en la región del Cáucaso Sur (Shulaveri Gora y Gadachrilli) brindan la evidencia arqueológica biomolecular más temprana para el vino de uva y la vitivinicultura (McGovern *et al*., 2017). En ellos se han encontrado restos de bitartratos, ácido tartárico, málico, cítrico y succínico en el interior de los fragmentos de dicho tipo de cerámica, así como con la evidencia arqueobotánica de polen de uva, almidón y restos epidérmicos asociados. Todo ello revela su uso como contenedores vínicos. Los procesos de restauración han revelado que su morfología era bastante similar a los de las actuales vasijas de fermentación, llamadas *quevri*, que tuvieron mucho mayor volumen, hasta unos 8.000 L (Paadín y Paadín, 2019) (*Figura 16*).

También en Georgia, las excavaciones realizadas en Uplistsikhe han revelado la presencia de semillas con una edad de unos 4.500 años, así como la presencia de lagares rupestres y bodegas excavadas en la roca, con hoyos para albergar los quevris (*Figuras 17* y *18*).

Llama extraordinariamente la atención que la palabra georgiana ვინო se lea /ẃi:nɘu/, voz de la que derivarían los vocablos *Vinum, Vino, Vinho, Wine*, etc. (Comunicación personal de Agnes Minnery y Marta Llamas). Lo cual parece reiterar, aún más, el carácter de centro de domestica-

Figura 13. Excavación en el tell de Shulaveri Gora (Georgia).

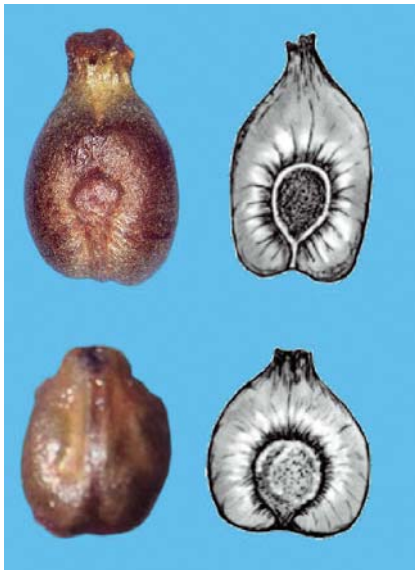

Figura 14. Diferencias morfológicas entre semillas cultivadas y silvestres.

Figura 15. Semillas cultivadas procedentes de Shulaveri Gora, con una antigüedad de 8.000 años. Museo Arqueológico de Tbilisi.

ción primario de la viticultura en la región geográfica que nos ocupa.

También en las tierras altas de Armenia existen pruebas de esa domesticación de la vid (McGovern, 2003; Forni, 2004; Haroutunian, 2005; Arroyo–García *et al*., 2006; Pelsy, 2010). Dicho país tiene una larga tradición en la elaboración de vino que supera los 6.000 años. De hecho, la bodega más antigua del mundo, hasta ahora, fue descubierta durante las excava-

Figura 16. Quervri primitivo. Su decoración imita racimos de uva. Museo Arqueológico de Tbilisi.

Figura 17. Lagar rupestre de Uplistsikhe.

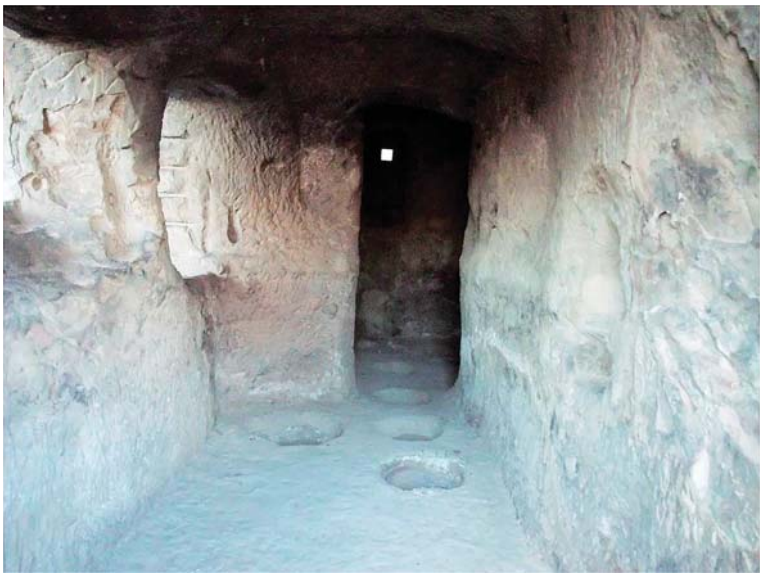

Figura 18. Bodega excavada en la roca. Pueden verse los hoyos para albergar los quevris.

ciones llevadas a cabo en 2007–2010, cerca del pueblo de Areni (provincia de Vayots Dzor) por un equipo internacional de arqueólogos armenios, estadounidenses e irlandeses. Este yacimiento arqueológico fue fechado alrededor del 4100 a.C. El complejo de cuevas "Areni–1" contenía un lagar, vasijas para la fermentación y almacenamiento de vino, tinajas, e incluso vasos cerámicos para beber (Wilkinson *et al*., 2012). Se encontraron también semillas con índices morfométricos intermedios entre silvestres y cultivadas y restos de racimos. Por su parte, el análisis químico de las tinajas mostró la presencia de residuos de vino, como los ya indicados en el caso de los proto–quevris, y, también, trazas de malvidina, un pigmento de uva propio de vinos rosados y tintos (Wilkinson, 2012; Smith *et al*., 2014) (*Figura 19*).

A título reivindicativo de tal actividad enológica de gran importancia histórica, cerca de la citada cueva, se celebra el primer sábado de octubre, desde 2009, el Festival de Vino de Areni. Este es un importante evento armenio en el que se recrea la forma ancestral de producción de vino, se consume comida típica y se bailan danzas tradicionales.

Figura 19. Recipientes para vino de la Cueva de Areni 1 (Armenia).

En Azerbaiyán se han descubierto en Uzerlitepé semillas cultivadas de la Edad del Bronce, con unos 3.500 años de antigüedad.

Los diversos análisis genéticos, en los que se compara material silvestre y cultivado de Transcaucasia confirman la relevancia de las poblaciones de vid silvestre del Cáucaso en el proceso de domesticación de esta vitácea (Zecca *et al.*, 2012; Pipia *et al.*, 2012; Dallakyan *et al.*, 2015; Riaz *et al.*, 2018). Es destacable la estrecha similitud de vides silvestres de Georgia con ciertas accesiones cultivadas aún en sus viñedos (Maghradze *et al.*, 2020).

El vocablo *gora* en otras lenguas

El nombre de ese lugar emblemático, Shulaveri Gora, significa literalmente en castellano *Colina de Shulaveri*. Resulta curioso que la palabra *gora* también signifique montaña o monte en idiomas eslavos. De hecho, en la península balcánica, Montenegro se llama, realmente, *Crna Gora* en su idioma vernáculo.

Una parte de la actual Georgia se llamaba en la antigüedad Iberia, como nuestra Península, nombre dado a los límites del mundo conocido por los griegos en la antigüedad (oikumene).

Existen ciertos vocablos georgianos que comparten algunas raíces con el euskera hablado en el País Vasco (Euskalherria) (Comunicación Personal de Xabier Kintana), tanto en territorio español como francés (Hegoalde e Iparralde, respectivamente). De hecho "*gora*", en ese idioma, significa "*viva*" o "*arriba*", en el sentido de vitorear a la persona o cosa que se menciona detrás, e igualmente se utiliza para designar algo "en alto" y "elevado". De la misma manera, la partícula "*goi–a*" hace referencia a la parte de arriba o superior de algo, o que esté situado en alto o más alto que otra referencia. Por eso Goierri significa tierra alta y Goikoetxea la casa de arriba, el primero como topónimo de una extensa comarca guipuzcoana de interior, y el segundo como apellido de procedencia.

No debe olvidarse que en este sentido podemos hacer mención al primer apellido de nuestro célebre pintor Francisco de Goya y Lucientes, nacido en Fuendetodos (Zaragoza), pero también con un origen vasco innegable, ya que *Goia* significa el alto. Ese Goya le llega de su tatarabuelo Domingo de Goya, nacido en el caserío Mantxolatxiki, situado en un barrio alto de la localidad de Zerain. Otra partícula relacionada con *gora*, pero de significado incierto, es el prefijo *gor–*, presente en la toponimia del occidente vasco, así, tenemos la sierra de nombre Gorobel, la aldea de Gordeliz y, en el límite entre Álava y Vizcaya, se encuentra el famoso monte Gorbea con idéntica raíz, a cuyos pies se conservan algunos restos de poblaciones de parras silvestres (Ocete *et al.*, 2004).

La vinificación en Quevri y el empleo de recipientes de cuerno como vaso

Los quevris son tinajas de cerámica que se encuentran generalmente soterradas en las bodegas, llamadas *marani*, con el fin de que la temperatura sea bastante estable durante la producción de vino. En estas tinajas, se producen los llamados *Vinos ámbar*, en el caso de que la uva fuese blanca o, bien, tintos. En ambos casos, tras el prensado de los racimos, se introduce en las tinajas el mosto con los hollejos, sin efectuar, en muchos casos, la labor de despalillado. La fermentación se realiza con levaduras autóctonas adheridas al hollejo de las uvas recolectadas.

Los operarios remueven la pasta más de diez veces al día durante el periodo de fermentación alcohólica, que se suele producir en un intervalo térmico de 14 a 19ºC, durante unos 20–40 días. El tiempo de maceración oscila bastante, dependiendo de múltiples factores. Por término medio, se suelen dejar uno o dos meses los tintos y hasta 6 meses los blancos (Bene *et al*., 2019).

En las catas llama increíblemente la atención de los aromas afrutados de esos vinos blancos y su buena acidez en boca y agradable postgusto, factores que dependen fundamentalmente de la variedad empleada y la climatología de cada año. Destacan los caldos elaborados con la clásica variedad 'Rkatsitely', la *explosiva* 'Kisi' y la *elegante* y escasa 'Khikhvi'), por su genuina y majestuosa boca y con una acidez volátil que oscila entre los 0,4 y 0,6 g/L. Para obtener esas características organolépticas, en el resto del mundo hay que recurrir a la refrigeración de la uva, a mantener la temperatura de fermentación controlada y al empleo de levaduras comerciales, así como a la adición de tartárico para incrementar la acidez. Tras la fermentación alcohólica, lógicamente se produce la maloláctica, generalmente sin control artificial.

A continuación, en las bodegas tradicionales, el quevri se cierra con una losa redondeada que se suele fijar a la boca de la tinaja con barro arcilloso. En primavera, normalmente en marzo o abril, de destapan y el vino se trasiega a otro quevri limpio. Cuando el proceso de crianza ha llegado a su fin, el vino no se suele someter a un enfriamiento y filtrado exhaustivo (Paadín y Paadín, 2019) (*Figuras 20, 21, 22* y *23*).

En los últimos años, este tipo de vino ha polarizado la atención internacional de enólogos, importadores, sumilleres, consumidores y periodistas. A ello ha contribuido decisivamente su reconocimiento como Patrimonio Cultural Inmaterial de la Humanidad desde 2014. Su sistema de producción, con ciertas similitudes al del conocido como *vinho de talha* (vino de tinaja) del sur de O Alentejo (Portugal), se ha copiado en varios países europeos.

La limpieza de los quevris se sigue haciendo, en pequeñas bodegas, con métodos tradicionales, raspando las paredes con ramas secas y corteza de cerezo y, a veces, empleando lechada de cal (Paaadín y Paadín, 2019).

En Transcaucásia está muy generalizado el uso de recipientes de cuernos para beber vino, tanto en el medio rural como en los actos festivos tradicionales. En la mayoría de los casos proceden de ganado vacuno. En Tbilisi, hay una plaza que se encuentra presidida por una estatua de bronce de un hombre portando un vaso de este tipo, se le conoce por "*tamada*" en georgiano; es decir, *el individuo que tiene la palabra en un banquete* o *lo dirige* (*Toast master*, en inglés). Su intervención provoca la contestación de otros comensales "*alaverdi*". Se trata de una copia de una pequeña figura del s. VII a.C encontrada en una excavación arqueológica en Vani. En las *Figuras 24–26* pueden, observarse, la citada estatua, uno de estos recipientes armenios con adornos en plata, con un grabado del Monte Ararat. También, una curiosa foto de Nikita Kruschev, el que fuera presidente de la URSS, con Fidel Castro, bebiendo en una visita a Georgia.

La tradición armenia de destilados

En la segunda mitad del s. XIX, varios industriales radicados en Yerevan comenzaron a preparar destilados al estilo francés, tipo Cognac. En 1899, Nikolái Tairov vendió su destilería al industrial ruso Nikolái Shustov, pasándose a llamar "Shustov e Hijos". La nueva empresa realizó multitud de innovaciones técnicas en el sistema de destilación, lo que incrementó la calidad del producto que comenzó a ser más valorado por

Figura 20. Fotografía de Vavílov junto a quevris. Foto cedida por VIR, San Petersburgo (Rusia).

Figura 21. Conjunto de quevris.

Figura 22. Interior de una bodega (*mariani*), donde se aprecian las bocas de los quevris.

los paneles de cata y lo introdujo en el mercado internacional.

En 1901, Shustov envió su brandy a un concurso parisino, donde el jurado le otorgó el "Grand Prix", pese al chovinismo galo imperante en aquella época. Como una importante excepción, dieron el privilegio especial a Shustov de vender el producto no como "*brandy*" genérico, sino bajo el apelativo de "*cognac*".

La citada empresa, tras una larga andadura, pasó a llamarse Yerevan Brandy Company. En 1998 pasó a formar parte del Grupo Pernod Ricard, líder mundial en la industria de las bebidas espirituosas *Premium*, que comercializa este producto armenio con la marca Ararat.

Otra importante empresa de bebidas alcohólicas, radicada también en Yerevan, es la Noy Wine, Brandy and Vodka Factory. En 2002 el grupo financiero liderado por Gagik Tsarukyan, renovó las instalaciones de la bodega y recuperó las antiguas tecnologías para la fabricación de sus destilados. Uno de sus productos estrella es el brandy Noy (Noé), con diferentes años de crianza, que tiene varios galardones internacionales. Desde 2006, esta empresa se ha convertido en suministradora oficial del Kremlin.

La gran biodiversidad de la vid cultivada en Transcaucasia y el problema de la erosión genética

Según Negrul (1946), el sur del Cáucaso incluye parcialmente dos de los tres principales grupos ecogeográficos de vid cultivada. Estos fueron denominados *proles* por dicho investigador

Figura 23. Tapadera de piedra que se sella a la tinaja con una pasta de arcilla mojada.

Figura 24. *Tamada.*

Figura 25. Vaso de cuerno vacuno con el Monte Ararat grabado en plata (Armenia).

soviético: *oriental y póntica*. La oriental está constituida por cepas con grandes bayas, propias para su consumo en fresco o pasificadas. La póntica por aquellas variedades domesticadas en los alrededores del Ponto Euxino, nombre dado por los griegos (Mar Negro actual), con características intermedias entre las anteriormente citadas y las de la zona central y occidental de Europa, que fueron cultivadas hasta los confines del antiguo Imperio austrohúngaro.

En Armenia, Azerbaiyán y Georgia, se cultiva una gran variedad de vides (Babayev, 1988; Unwin, 1991; Chkhartishvili y Maghradze, 2012; Melyan y Gasparyan, 2012), tanto hermafroditas como femeninas, estas últimas en mucha menor proporción, llegando todo el conjunto a contabilizar más de 1.300 variedades (Maghradze *et al*., 2012).

Ya, en los años noventa del siglo pasado, el banco de germoplasma de la Colección Central Nacional, situada, en el Valle de Ararat albergaba 600 variedades autóctonas (Melyan and Gasparyan, 2012 *et al*., Aroutiounian *et al*., 2015; Nebish *et al*., 2017; Melyan *et al*., 2019). De ellas, en torno a un 5% de las mismas son ejemplares femeninos. En el caso de Georgia, con unos 550 cultivares, aproximadamente, el 10% son vides femeninas.

Su número exacto es muy difícil de concretar, dada la existencia de numerosas homonímias y sinonímias, tratándose, además, de países con tres alfabetos diferentes.

Debe destacarse la presencia de variedades con diversos grados de tolerancia a la salinidad y la sequía (Musayev y Huseynova, 2016).

Aquellas variedades cultivadas más hacia el oeste, tanto en Europa central como occidental, fueron ubicadas en la *prole occidental*. Estas vides forman bayas de menor tamaño, con menor volumen de pulpa y, por tanto, con una mayor proporción de hollejo, lo que hace que sean las idóneas para vinificación. Se cultivan desde Europa central hasta la Península Ibérica. Más tarde, se exportaron hacia las colonias europeas situadas en Islas Canarias, América, África Asia y Oceanía. Con ellas se elaboran la mayoría de los vinos actuales, donde progresivamente, debido a la globalización del comercio mundial, se obtienen, en la mayoría de los casos, de viníferas francesas. No de variedades–población de las mismas, sino de clones seleccionados que incrementan el terrible fenómeno de erosión genética del viñedo, reduciendo drásticamente su biodiversidad genética. Este hecho constituye un grave problema a la hora de enfrentar la producción de uva de calidad frente al patente desafío de cambio climático y la posible aparición de nuevas plagas y enfermedades.

En la actualidad, a nivel mundial, se contabilizan unas 1.500 variedades de vid que se siguen cultivando manteniendo en los viñedos. Estos han pasado de contener mezclas de distintas variedades y variantes somáticas de las mismas a estar constituidos por clones únicos, reduciéndose de forma drástica su agrodiversidad.

Hay que señalar con énfasis alarmante que 16 variedades ocupan el 50% de la superficie mundial del viñedo actual (Anderson y Aryal, 2017).

Figura 26. Nikita Kruschev, el que fuera presidente de la URSS, con Fidel Castro en una visita a Georgia. Mercadillo de antigüedades de Tbilisi.

Entre ellas se encuentran la variedad blanca 'Airén', propia de La Mancha, y el 'Tempranillo'. Como puede deducirse, las variedades locales de cada región han sido desplazadas en parte por otras que se han expandido internacionalmente debido a la globalización de los mercados (Marsal *et al.*, 2017).

Abundando a lo expuesto en el párrafo precedente, por ejemplo, la variedad 'Shyrah' se va extendiendo por el sur de Europa, como es el caso de Andalucía, para dar una capa de color más intensa a los vinos tintos, no alcanzable con variedades tradicionales. De ahí, la necesidad de salvaguardar las variedades tanto silvestres como minoritarias de cada zona vitivinícola.

En la zona transcaucásica, como centro principal de domesticación de la vid, existe una gran variedad de clorotipos, principalmente B, C y D (Comunicación Personal de Javier Ibáñez), mientras que en las viníferas europeas, cultivadas desde su zona central a la occidental, domina claramente el clorotipo A, el único encontrado en las parras silvestres ibéricas (Arroyo *et al.*, 2006; De Andrés *et al.*, 2012).

La conservación del agrotipo silvestre en refugios de zonas meridionales de Europa pudo permitir a estas regiones ser centros secundarios de domesticación (Arroyo *et al.*, 2006; Maras *et al.*, 2020). No obstante, sus poblaciones son relictas y el número de parras conservadas en el medio natural se reduce progresivamente, debido a una larga suma de impactos antrópicos (Arnold, 2002) y a la falta de protección legal en la mayoría de los países de la Unión Europea, como en el caso de España (Ocete *et al.*, 2014). Debido a lo cual, la Organización Internacional de la Viña y el Vino (OIV), en su resolución 424/2010, emanada de su Asamblea General, recomienda *«emprender cuanto antes amplias campañas de prospección destinadas a catalogar el material salvaje y de cultivo en peligro de extinción e identificar, cuando proceda, los genotipos originales o aun no descritos ni caracterizados».*

Debe reseñarse que, hoy en día, la vid se ha llegado a convertir en el principal cultivo frutal de amplia difusión e importancia comercial a nivel mundial (Vivier y Pretorius, 2002).

De acuerdo con todo lo expuesto, resulta de gran importancia la conservación de los ejemplares silvestres como los tradicionalmente cultivados, en sus hábitats naturales, viñedos y bancos de germoplasma.

Dedicatoria

Los autores desean dedicar este artículo a la memoria de un gran experto en la viticultura de Armenia, compañero y amigo, el Dr. Gagik Melyan, recientemente fallecido, perteneciente a la Armenian Academy of Viticulture and Wine–making, ubicada en Ereván (Armenia), con el que

varios de ellos tuvieron relación a través de Proyecto de la Unión Europea Cost Action FA1003 – GRAPENET. East–West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding (*Figura 27*). •

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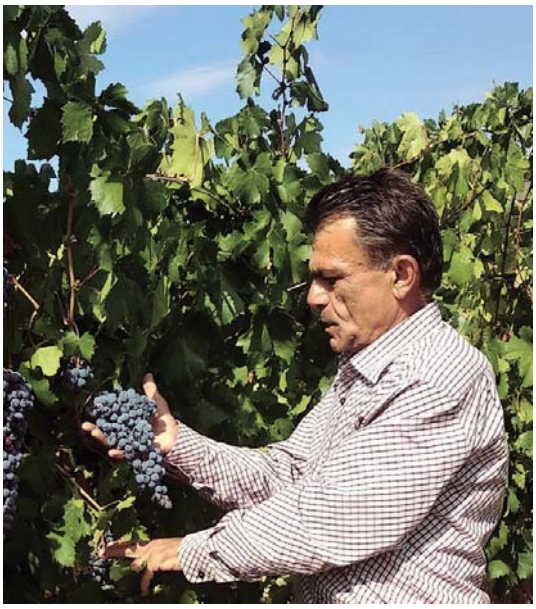

Figura 27. Fotografia del Dr. Gagyk Melian, gran experto en Viticultura, a quien va dedicado el presente artículo.

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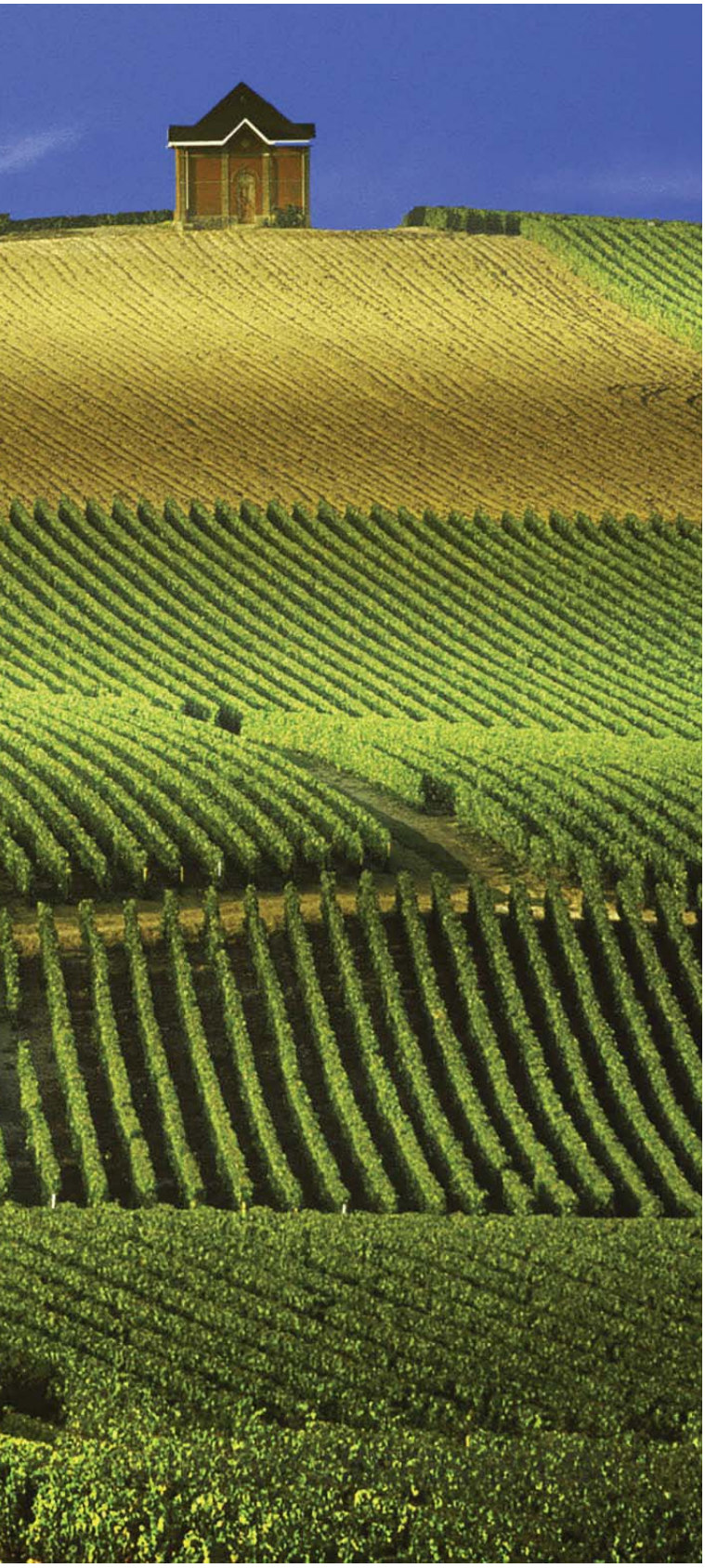
El origen de la viticultura

Cepas y Vinos. Mayo. 2020: 36-42

El origen de la viticultura



El presente artículo trata de ofrecer una visión panorámica sobre el origen de la Viticultura. Parte de una idea de la cantidad de especies silvestres de vid que existen en el planeta, para centrase en la euroasiática, ya que constituye el parental de las variedades de cultivo que han sido seleccionadas y multiplicadas por el hombre desde el periodo Neolítico hasta la actualidad.

Sobre dicha vitácea se da una idea general de su ecología, sus principales características ampelográficas (descripción botánica), usos tradicionales y sobre los impactos antrópicos que han reducido drásticamente sus poblaciones. Asimismo, se comenta como empezó a llevarse a cabo su proceso de domesticación.

Por otra parte, se destaca la importancia de estas parras silvestres como recurso fitogenético para intentar paliar parte de la problemática inherente al cambio climático. Actualmente, asistimos a una alarmante reducción del número de variedades cultivadas. Además, hay que tener en cuenta que de éstas, únicamente se plantan determinados clones comerciales, lo que provoca una pérdida de biodiversidad.

La diversidad de las vides silvestres

La bibliografía recoge que a nivel mundial existen del orden de unas 70 especies silvestres, pertenecientes a la familia Vitaceae y dentro de ella, al género Vitis, donde todas sus especies contienen 38 cromosomas. La mayor parte de ellas se encuentran en Estados Unidos y China.

Las poblaciones de vid silvestre euroasiática aparecen entre la Península Ibérica y el Macizo del Hindhu Kush, entre las latitudes correspondientes a los paralelos 30-31 (río Ourika, situado a los pies de la Cordillera del Atlas, Marruecos) y 49-50 (en los alrededores de Lwfdishaffen, en las orillas del río Rin, Alemania). Su nombre científi o es Vitis vinífera L. subespecie sylvestris (Gmelin) Hegi. Se trata de una subespecie dioica; es decir contienen ejemplares con flo es masculinas y otros con flo es femeninas. Las vides cultivadas obtenidas a partir de este parental silvestre se incluyen dentro de la subespecie Vitis vinifera L. subespecie sativa (DC.) Hegi, y son mayoritariamente hermafroditas. Se emplean tanto como uva de mesa, para pasificación, preparación de mostos, vinificación y ob ención de cosméticos.

Características ampelográficas

Las plantas masculinas suelen tener las hojas más lobuladas y pequeñas que las femeninas. Sus racimos florales son de mayor tamaño que los femeninos y exhalan un olor característico. Sus flores, normalmente, contienen 5 estambres erguidos y carecen de gineceo (órgano femenino) (Tipo I). No obstante, en una proporción muy inferior, pueden verse parras masculinas con un gineceo poco desarrollado (Tipo II).

Las flores femeninas contienen un gineceo bien patente y 5 estambres reflejos (curvados hacia abajo) (Figura 2).

El polen masculino de los estambres es muy abundante y tricorporado; es decir, con orificios para el tubo polínico por donde se evacuan los gametos para la fecundación de las flores del sexo opuesto. Por el contrario, el polen femenino es escaso y carece de

Figura 1. Ejemplares silvestres de la playa de Atxasbiribil (Vizcaya)

Ecología

Las parras silvestres euroasiáticas crecen en bosques de ribera de ríos y arroyos, principalmente. También en llanuras de inundación de grandes ríos, en zonas montañosas con alta precipitación, acantilados marinos y en suelos arenosos (arenosoles) como los de la desembocaduras del Guadalquivir y del Danubio.

Se trata de lianas hidrófilas que trepan mediante sus zarcillos sobre la vegetación circundante, árboles y arbustos, para obtener una intensidad lumínica adecuada (Figura 1).

dichos orificios, por lo que no pueden autofecundarse. Debido a ello, la polinización es cruzada.

La inmensa mayoría de las plantas femeninas producen racimos de uvas tintas con tamaño inferior a 1 cm de diámetro, con la pulpa no coloreada. Contienen entre 1 y 4 semillas. Éstas son más redondeadas y con un pico mucho más corto que las cultivadas. Por este motivo, en los yacimientos arqueológicos se puede averiguar si la gente de una determinada época consumía vid silvestre o cultivada (Figura 3).

Usos tradicionales de las parras silvestres

Sus bayas fueron empleadas como alimento humano en diversas épocas, desde el Paleolítico hasta la posguerra de la última guerra civil española. Además constituyeron la materia prima de las primeras vinificaciones (Figura 4). Su uso para producir vino se ha mantenido en Cerdeña hasta casi finales del siglo anterior. Allí los pastores elaboraban el llamado vino de vulpa (vino de zorra). Hasta dicha fecha, aproximadamente, se ha producido vinagre casero en Sierra Morena, Parque Natural de Cazorla, Segura y Las Villas y en la Serranía de Grazalema (Andalucía). El mosto, debido a su alto contenido en ácido tartárico, ha sido empleado para evitar la aparición de fisuras durante la cocción de las piezas cerámicas desde la Edad del Bronce.

La farmacopea española contiene muchas referencias a remedios medicinales a base de extractos y órganos de vid silvestre.

Los sarmientos tienen una gran flexibilida , por dicha cualidad se han empleado para la fabricación de maromas, empleadas incluso por la Armada española. Asimismo, hemos visto subir a gente de Barbate y alrededores (litoral gaditano) al Parque Natural de los Alcornocales (Cádiz), con el fin de recoger sarmientos para confeccionar los aros de las nasas de pesca para la langosta.

En varias necrópolis aparecen semillas de parras silvestre y/o cultivadas, ya que las ofrendas de racimos eran muy comunes en los rituales funerarios, desde la Cultura argárica hasta la época paleocristiana.

Impactos antrópicos

La reducción de las poblaciones de parras silvestres se ha debido a varias causas, que enumeramos a continuación. Entre ellas se encuentra la limpieza de bosques de ribera y establecimiento de huertos o plantaciones de chopos y eucaliptos, principalmente. Obras públicas, como el ensanche o nuevo trazado de vías de comunicación, con la correspondiente y periódica limpieza de las cunetas, así como indencios. También, la construcción de embalses. En este caso hemos contemplado la enorme pérdida de parrales silvestres en el río Guadiana y afluen es en el Alentejo portugués, tras la construcción del importante y necesario pantano de Alqueva.

Cabe destacar que la llegada de vides de origen norteamericano para remediar la infestación fil xérica desde el último cuarto del s. XIX, usadas tanto como portainjertos o híbridos productores directos, ha provocado la infección de nuestros contingentes silvestres por el oídio y mildiu. En el medio natural, las vides no son atacadas por la fil xera ya que crecen en suelos muy húmedos o arenosos.

Las vitáceas americanas citadas se han ido escapando de los viñedos, convirtiéndose en plantas invasivas que están desplazando a nuestras parras silvestres autóctonas de sus hábitats naturales, principalmente de los bosque de ribera.

La pérdida de parras silvestres autóctonas, acarrea también la de parte de la vegetación acompañante y la biocenosis de artrópodos predadores y parasitoides que regulan las poblaciones de fi ófagos que atacan a esta vitácea. La diversidad y abundancia de estos últimos es mucho mayor que la registrada en los viñedos, debido al uso de biocidas. Paralelamente, desaparecen los hongos de las micorrizas arbusculares asociadas a su sistema radicular y buena parte del conjunto de levaduras autóctonas.

Por todo lo expuesto en este apartado, actualmente, la vid silvestre euroasiática constituye un taxón realmente amenazado en toda la amplia zona geográfica donde habitaba.

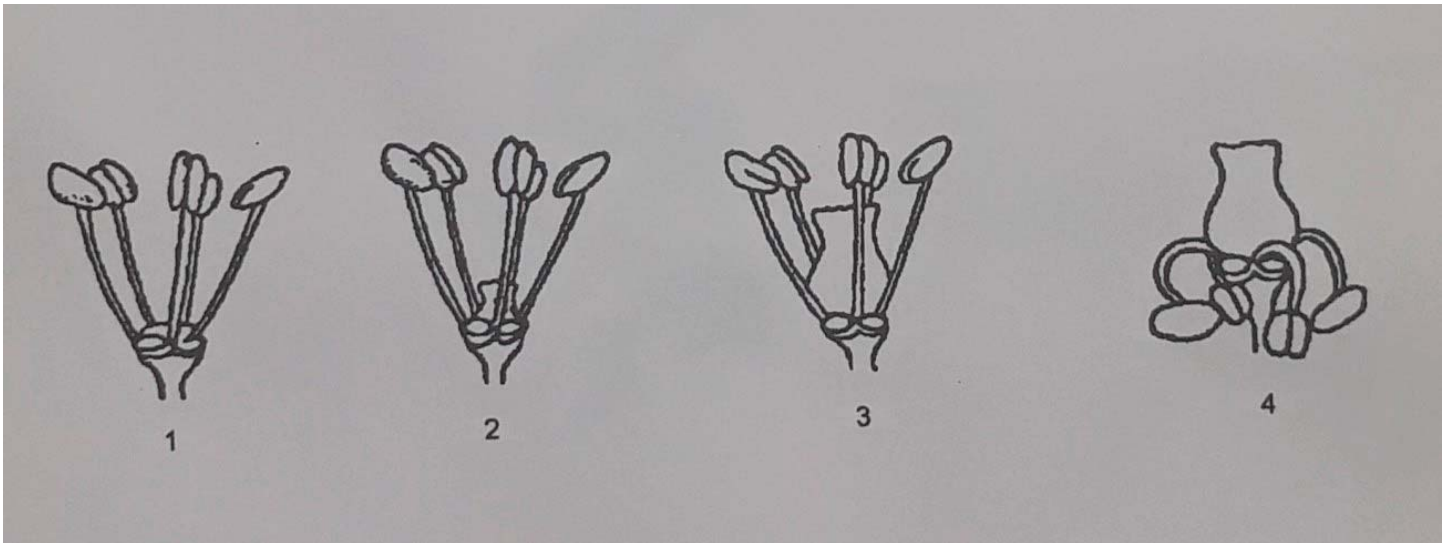


Figura 2. 1- Flor silvestre masculina tipo I; 2-Flor silvestre masculina tipo II; 3- Flor hermafrodita de vid cultivada; 4- Flor silvestre femenina con estambres reflejos (Esquemas del ódigo de descriptores 151 (2009) de la OIV (Organización Internacional de la Viña y del Vino).

El proceso de domesticación

Dentro de las poblaciones de parras silvestres se producen algunas mutaciones de ejemplares masculinos que conllevan a la aparición de ejemplares hermafroditas autofecundables. Éstos exhibían un mayor nivel de cuajado, que no escapó a la observación de los grupos humanos recolectores de frutos. Por tal motivo, sus sarmientos fueron elegidos para realizar plantaciones fuera de los hábitats naturales. Había nacido la Viticultura (Figura 2).

Varios trabajos multidisciplinares avalan la idea de que el cultivo de la vid se irradió a partir del territorio del llamado Triángulo de la Uva fértil de Vavilov, donde dada su orografía, existieron varios refugios durante las glaciaciones del Cuaternario para las parras silvestres y otros parentales de frutales. Dentro de dicha zona geográfica, se en- cuentra el Monte Ararat, el lugar donde según la tradición judeo-cristiana, inspirada en el Libro del Génesis, encalló el arca de Noé. Dentro del citado triángulo, en la República de Georgia se encuentra el yacimiento arqueológico de Shulaveri Gora. En el nivel correspondiente a hace unos 8.000 años se aparecieron las primeras semillas de vid cultivada, según los datos actuales. No muy lejos de allí, en el sur de Armenia, se encuentra la cueva de Areni-1, donde aparecieron una prensa, una cuba de cerámica, así como restos de racimos de uva y semillas con una antigüedad de unos 6200 años. Otros hallazgos arqueológicos y la enorme biodiversidad de vides cultivadas y silvestre permiten concluir que el sur del Cáucaso (Transcaucasia) constituye la cuna de la Viticultura.

No obstante, la conservación del agrotipo silvestre en refugios de zonas meridionales de Europa, incluida nuestra Península, pudo permitir a estas regiones ser centros secundarios de domesticación. Ello se basa en el estudio de los clorotipos realizado mediante análisis de ADN de los cloroplastos en variedades de cultivo consideradas autóctonas que ha revelado que más del 70% de las viníferas ibéricas contienen el clorotipo A, al igual que las poblaciones silvestres de la zona. En cambio, en la zona Transcaucásica son los clorotipos C y D los que abundan en una mayor proporción, tanto en variedades de cultivo como en las poblaciones silvestres. Es posible que las variedades importadas por fenicios, griegos, romanos y cartagineses, pudieron hibridarse con las poblaciones silvestres locales. Dicho patrimonio genético se fue enriqueciendo, posteriormente, con las aportaciones de los árabes y de la Ruta Jacobea.

Figura 1. Ejemplares silvestres del río Esca (Navarra-Zaragoza).

La vid silvestre como recurso fitogenético

En todos los países vitivinícolas, el número de variedades tradicionales cultivadas se redujo bastante tras la reconstrucción del viñedo por causa de la filoxera. También ha contribuido, las normativas internacionales, nacionales y autonómicas, así como la creación de las diversas Indicaciones de Origen Protegidas y de las Denominaciones de Origen. Además cabe resaltar que de cada variedad, hay un número de clones muy reducido en el mercado, lo que prácticamente anula la diversidad genética intravarietal de las nuevas plantaciones, afectadas por el creciente proceso de erosión genética. La prueba la tenemos en que pueden existir entre 5.000 y 10.000 variedades de cultivo, a nivel mundial. De ellas, unas 1.500 se siguen manteniendo en los viñedos. Hay que resaltar, con agonía, que en las zonas vitícolas, únicamente 16 variedades ocupan el 50% de la superficie mundial. Donde se encuentran las variedades españolas Airén y Tempranillo.

Los viñedos han pasado de contener mezclas de distintas variedades y variantes somáticas de la misma variedad a estar constituidos muchos de ellos por clones únicos, reduciéndose de forma drástica su biodiversidad. Es decir, la base genética sobre la que actúa la selección natural. Este dramático hecho puede constituir un grave problema de cara al cambio climático y a la aparición de nuevas plagas y enfermedades.

Las parras silvestres exhiben una gran biodiversidad, gracias a su reproducción sexual. Generalmente presentan importante resistencia al encharcamiento y a la caliza activa. Estas son dos características muy interesantes para la obtención de nuevos portainjertos, hibridando con los actuales o con especies de origen norteamericano. Las microvinificaciones realizadas con ellas muestran que los vinos obtenidos poseen una buena acidez (pH ligeramente superior a 3) e intensidad de color (entre 11 y 26), dos importantes cualidades a la hora de generar nuevas viníferas para enfrentarse al cambio climático. La concentración de alcohol varía extraordinariamente, dependiendo de las zonas (entre algo más de 5 grados hasta 14,5). Sería interesante valorar la concentración de estilbeno en los sarmientos lignificados tras la caída de la hoja. Ya que este producto natural puede ejercer una protección de los vinos sin los problemas alérgicos que puede desarrollar la adición de metabisulfito potásico para su conservación.

Pese a todo lo expuesto, se denuncia que España, país que alberga el mayor viñedo del mundo (unas 960.000 ha), carece de una protección específica para la conservación de la vid silvestre en el medio natural, tanto a nivel estatal como de sus comunidades autónomas. Para evitar su extinción, algunos ejemplares se guardan en bancos de germoplasma de Cádiz, Madrid, Badajoz, La Rioja, Álava y Vizcaya.

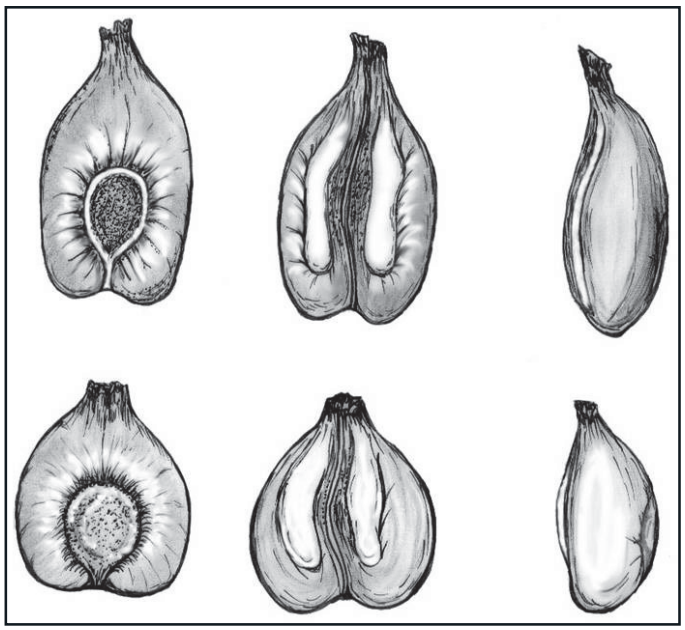


Figura 3. Parte superior: esquema de semillas de vid cultivada. Parte Inferior: esquema de semillas de vid silvestre femeninas.

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Figura 4. Racimo de vid silvestre. Nótese la diferencia del grado de maduración de las bayas, Shirikhevi (Georgia).

Il germoplasma di vite del Caucaso fonte di resistenza e qualità delle uve

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Il valore viticolo-enologico e la presenza di promettenti fonti di resistenza nei confronti delle malattie della vite incoraggiano ad approfondire le ricerche per la caratterizzazione e la valorizzazione delle numerose risorse genetiche della vite nei Paesi Caucasici.

L'origine della biodiversità della vite domestica

Vi è una ricca documentazione bibliografica, basata su analisi archeologiche e archeobotaniche, che individua nella regione compresa tra i Monti Tauros, in Anatolia orientale, e il Caucaso meridionale, localizzato tra il Mar Nero e il Mar Caspio, la culla di origine della vite domestica e dello sviluppo dell'enologia (Mac Govern, 2003). In particolare, nell'Alta Valle dell'Eufrate sono stati ritrovati i più antichi resti fossilizzati di vinaccioli, datati nell'VIII millennio a.C., in un contesto archeologico compatibile con la vinificazione di significative quantità di uva, che verosimilmente era però di vite selvatica (*Vitis vinifera* L. subsp. *sylvestris* (Gmel.) Hegi).

Le più antiche attestazioni di vinaccioli di vite domestica (*Vitis vinifera* L. subsp. *sativa* (DC.) Hegi) sono invece state rinvenute nell'attuale territorio della Georgia, nell'area interessata dalla cultura Shulaveri-Shomu che dalla metà del VI millennio all'inizio del V millennio a.C. (tardo Neolitico, inizio età del rame) era diffusa in tutto il Caucaso meridionale (odierne Georgia, Azerbaigian e Armenia). In questa regione, dunque, avvenne la domesticazione della vite e la sua successiva diffusione sia verso le regioni mediorientali, sia verso la penisola anatolica.

La diffusione della vite domestica avvenne congiuntamente con le tecniche di vinificazione. Da questo punto di vista ebbe probabilmente maggiore importanza una cultura sviluppatasi, sempre in Georgia, successivamente (III millennio a.C.); si tratta della cosiddetta cultura transcaucasica precoce ("Early Transcaucasian Culture") che dal Caucaso si diffuse mediante migrazioni in tutta l'area medio-orientale, fondando insediamenti che mantennero molto a lungo, almeno fino alla metà del II millennio a.C., connotati culturali propri e fondati in particolare sulla produzione viti-vinicola (Batiuk, 2013). La lunga tradizione viti-vinicola georgiana è sottolineata, non senza orgoglio, nel motto associato al logo della National Wine Agency georgiana che recita "8000 vintages" ("8000 vendemmie").

La riscoperta delle risorse genetiche caucasiche

Tenendo presente, dunque, la cronologia della diffusione della viticoltura dal Caucaso verso Occidente, evidenziata nella mappa della figura 1, è facile comprendere come il germoplasma viticolo caucasico, rispetto a quello dell'Europa occidentale, abbia una storia evolutiva molto più

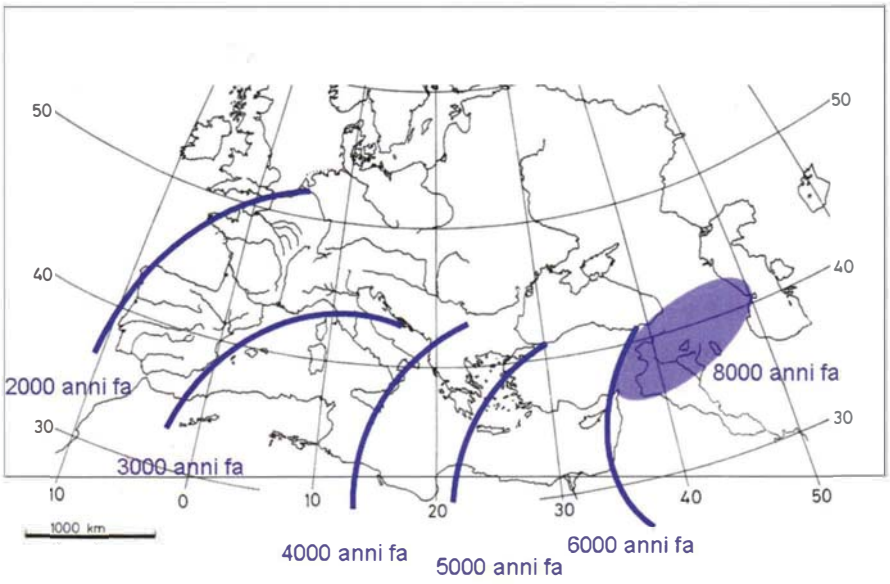


Fig. 1 - Mappa relativa alla cronologia della domesticazione e della diffusione della vite domestica.

▲ *Fig. 2 - Sintomi osservati in vigneti georgiani affetti da legno nero: sintomi gravi (alterazioni fogliari e disseccamenti dei grappoli; forte perdita di produzione) osservati sulla cv Chardonnay (A); sintomi lievi (leggere alterazioni fogliari; nessuna perdita di produzione) osservati sulla cv. Rkatsiteli (B) e Tsitska (C). Le analisi molecolari hanno rivelato la presenza dello stesso tipo di fitoplasma nelle viti in figura.*

lunga. C'è inoltre da notare come le condizioni climatiche del Caucaso siano molto differenziate, variando dalla zona a clima subtropicale prossima al Mar Nero, alle zone con clima propriamente continentale delle aree centrali e dell'altopiano armeno, fino a quelle con clima alpino delle pendici montane. Durante gli ottomila anni di storia della viticoltura caucasica, e nei diversi contesti pedo-climatici, è ragionevole ritenere che siano stati selezionati vitigni molto differenti da quelli coltivati in Occidente e con un grado di diversità genetica più elevato. In effetti, recenti lavori di analisi genetica (Imazio *et al.*, 2013; De Lorenzis *et al.*, 2015), condotta su consistenti campioni di accessioni maggiori e minori del germoplasma caucasico, proveniente da Georgia, Armenia ed Azerbaigian, e del Mar Nero settentrionale proveniente dalla Moldova, hanno messo in evidenza come effettivamente il "pool" genetico caucasico sia diverso rispetto a quello del germoplasma occidentale e come nel Caucaso il germoplasma azero identifichi una popolazione genetica ulteriormente differenziata da quella georgiana e armena. L'analisi genetica ha messo in luce un'elevata variabilità all'interno della popolazione di vitigni caucasici, evidenziata anche dall'assenza di relazioni strette di parentela fra le accessioni. Tali relazioni sono invece assai frequenti nell'ambito del germoplasma occidentale.

Grazie all'impulso dato dalle attività intraprese, a partire dal 2003, nell'ambito di una serie di progetti internazionali che hanno poi dato origine ad una "Cost action", coordinata da uno degli autori della presente nota

▲ *Fig. 3 - Particolare della collezione ampelografica presso il Centro di Saguramo del Scientific-Research Center of Agriculture (SRCA) presso Tbilisi in Georgia.*

(Failla, 2015) e denominata "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding", disponiamo ora di molte informazioni sulle caratteristiche del germoplasma caucasico.

Fenotipizzazione

Le indagini hanno coinvolto diverse centinaia di accessioni georgiane, armene ed azere, fenotipizzate in particolare per la fenologia, le caratteristiche del frutto, la resistenza alle malattie fungine (peronospora e oidio in particolare), la presenza di giallumi da fitoplasma (legno nero in particolare). I dati raccolti hanno confermato le attese in relazione alla presenza di caratteri fenologici, qualitativi e di resistenza di grande interesse.

In particolare, le accessioni georgiane sono tipicamente a maturazione tardiva o molto tardiva (Maghradze *et al.* 2012; Rustioni *et al.*, 2014); questo carattere è ovviamente di grande interesse per l'adattamento della viticoltura al cambiamento climatico. I vitigni a maturazione tardiva negli ambienti più caldi sfuggono infatti agli effetti negativi degli stress estivi e raggiungono più facilmente buoni profili di maturità tecnologica e fenolica rispetto a quelli precoci.

La fenotipizzazione delle caratteristiche eno-carpologiche delle accessioni del germoplasma georgiano e armeno (Abashidze *et al.*, 2015; Margaryan *et al.* 2015; Rustioni *et al.*, 2014) ha confermato la qualità enologica sia delle caratteristiche carpologiche, legate alla dimensione medio-piccola delle bacche e dotate di bucce consistenti, sia delle caratteristiche di ma-

▲ *Fig. 4 - Semenzali in corso di selezione per la resistenza a peronospora presso le serre site a Tavazzano (Lo) dell'Az. didattico-sperimentale Francesco Dotti dell'Università di Milano.*

▲ *Fig. 5 - Test di inoculazione con oidio su accessioni caucasiche presso le serre site a Tavazzano (Lo) dell'Az. sperimentale dell'Università di Milano.*

turità fenolica e tecnologica. Test di micro- e meso-vinificazione, in corso sia in Georgia, presso il Centro di Saguramo del Scientific-Research Center of Agriculture (SRCA), sia in Italia, presso alcune aziende dove sono state realizzati impianti sperimentali con vitigni georgiani, hanno consentito di valutare più compiutamente il potenziale enologico di numerose accessioni, confermando le elevate doti enologiche per struttura dei vini, eleganza, ricchezza e diversità negli aromi varietali.

Le resistenze

Accanto alle doti agronomiche ed enologiche il germoplasma caucasico si è rivelato anche fonte potenziale di resistenze e/o tolleranze ai patogeni più dannosi per la vite: peronospora, oidio e fitoplasma del legno nero. Le analisi sistematiche sulla suscettibilità di un campione di 94 vitigni georgiani all'agente della peronospora sono iniziate nel 2012 nella collezione ampelografica del Polo didattico e di ricerca dell'Università di Milano, presso l'Az. Riccagioia sita a Torrazza Coste (Pv). Lo "screening" è stato effettuato sia in pieno campo, ove erano stati sospesi i trattamenti antiperonosporici, sia in laboratorio, mediante inoculazioni su dischetti fogliari in condizioni controllate. Alle 94 accessioni allevate in pieno campo si sono aggiunte, a partire dal 2013, più di 200 altre accessioni introdotte in Italia da Georgia, Armenia, Azerbaigian, Moldova, Russia e Ucraina, nell'ambito delle attività previste dal progetto Innovine "Combining innovation in vineyard management and genetic for a sustainable European Viticulture", un progetto collaborativo europeo nato dalla Knowledge Based Bio-Economy (KBBE), lanciato in febbraio 2013 e che si completerà nel corso di un periodo di 4 anni. Tali accessioni sono allevate in vaso in condizioni di quarantena presso le serre site a Tavazzano (Lo) dell'Azienda didattico-sperimentale Francesco Dotti dell'Università di Milano.

Lo "screening" delle accessioni allevate in collezione ha messo in luce un'ampia variabilità nella suscettibilità delle accessioni a peronospora, evidenziando comunque, nelle condizioni di campo, la presenza di un numero significativo di accessioni che nel quadriennio 2012–15 hanno comunque fatto riscontrare ridotti indici di infezione su foglie e grappoli. Nei test *in vitro* su dischetti fogliari le accessioni si sono invece dimostrate in generale suscettibili, con alcune notevoli eccezioni (Bitsadze *et al.* 2015). In modo particolare, il vitigno Mgaloblishvili N. ha sempre manifestato livelli di resistenza comparabili al controllo resistente di *Vitis x labruscana*. Anche nell'ambito del materiale in quarantena sono state individuate alcune accessioni che nelle condizioni di test *in vitro* hanno mostrato indici di infezione ridotti e comparabili con quelli dei controlli delle viti resistenti.

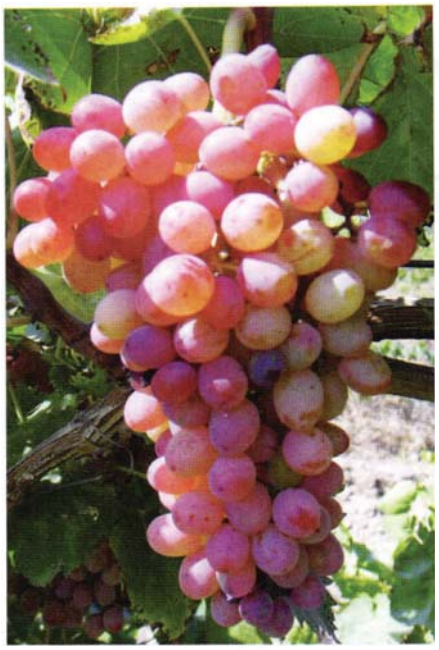
In seguito ai primi risultati conseguiti abbiamo proceduto alla costituzione di progenie di Mgaloblishvili N. per incrocio (con Pinot nero e Barbera), autofecondazione e libera impollinazione. Le progenie da autofecondazione, a differenze delle altre due tipologie, hanno segregato individui tolleranti/resistenti. Su questi materiali sono in corso approfondite analisi fenotipiche e genotipiche, in collaborazione con il laboratorio del Dipartimento di Genomica e Biologia delle piante da frutto della Fondazione

Edmund Mach (San Michele all'Adige – Tn), al fine di individuare i marcatori molecolari associati ai tratti di resistenza. Le caratterizzazioni fenotipiche hanno messo in evidenza come la resistenza individuata sia molto probabilmente di tipo multigenico e quindi basata sulla sintesi di barriere fisiche, come l'apposizione di callosio e lignina e la produzione di sostanze con proprietà antifungine quali composti fenolici, proteine "pathogenesis related", gli enzimi connessi allo scoppio ossidativo (perossidasi).

Oltre alla resistenza/tolleranza alla peronospora, nell'ambito del materiale caucasico, e specificatamente di quello proveniente dall'Azerbaigian, sono state individuate numerose accessioni con ridotta suscettibilità nei confronti dell'agente eziologico dell'oidio. La presenza del carattere di resistenza all'oidio, nelle accessioni di *Vitis vinifera* caucasica, oltre che osservata per via fenotipica, è stata confermata dalla presenza dei marcatori molecolari ad essa associati (Hoffman *et al.*, 2008; Riaz *et al.*, 2013).

Infine una serie di indagini di campo effettuate in Georgia, dopo avere rinvenuto la presenza di *Candidatus Phytoplasma solani*, agente eziologico di legno nero (Quaglino *et al.*, 2014), ha consentito di evidenziare come la maggior parte delle varietà georgiane, quando infette dall'agente del legno nero, mostrino sintomi moderati o lievi, mentre nelle stesse condizioni le cultivar internazionali hanno mostrato sintomi gravi (Fig. 2). La caratterizzazione molecolare del fitoplasma nelle viti georgiane ha rivelato la presenza di 11 tipi diversi, uno solo dei quali già noto, la cui presenza nelle diverse cultivar con una diversa intensità di sintomi suggerisce una diversa suscettibilità delle singole cultivar georgiane al legno nero (Quaglino *et al.*, 2015).

▲ ***Fig. 6 - Il grappolo a maturità del vitigno georgiano Mgaloblishvili.***



▲ ***Fig. 7 - Il grappolo a maturità del vitigno azero Agdam Gyulabisi.***

Chi desiderasse può scaricare al link: http://pub.jki.bund.de/index.php/VITIS/issue/view/983 una ampelografia (Maghradze *et al.* 2012) recentemente pubblicata e dedicata ad una selezione di quasi 300 cultivar di particolare importanza storico-geografica del germoplasma caucasico.

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