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SSR and SNP genetic profiling of Armenian grape cultivars gives insights into their identity and pedigree relationships

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ABSTRACT

The South Caucasus is recognised as the primary *Vitis vinifera* L. (grapevine) domestication centre and has a high diversity of wild and cultivated grapevines. Archaeological findings indicate that winemaking activities have existed in Armenia for more than 6,000 years, viticulture being one of the most important activities of the modern Armenian agricultural sector. Despite this relevance, some grapevines in local collections have not yet been properly identified, thus hindering the efficient conservation, characterisation and eventual use of autochthonous genetic resources. In the present study, a combined SNP and SSR profiling strategy was used for the genetic identification of a series of grapevine accessions from the Grape Collection of the International Academy of Viticulture and Winemaking in Nalbandyan, presumed to be autochthonous Armenian varieties. The results provided useful information for the correct identification of these genetic resources, revealing multiple cases of synonyms, homonyms and misnames. The genetic data made it possible to confirm the pedigree proposed for some of the cultivars identified in this study and to clarify the origin of others. In addition, we propose, for the first time, a series of new trios and duos involving autochthonous Armenian grapevines. The singularity of this genetic pool compared to other Western and Central European varieties, as well as the potential novel sources of variability in traits of interest (e.g., seedlessness) that were found, highlight the importance of improving knowledge of the Armenian grapevine genetic pool.

KEYWORDS

Grapevine, homonyms, genetic identification, misnames, Simple Sequence Repeat (SSR), Single Nucleotide Polymorphism (SNP), synonyms

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/4815

INTRODUCTION

The South Caucasus is acknowledged as the primary grapevine (*Vitis vinifera* L.) domestication center (This *et al.*, 2006). The genetic characterisation of grapevines from this region indicates the uniqueness and singularity of this germplasm when compared to Western genetic pools (De Lorenzis *et al.*, 2015; Imazio *et al.*, 2013; Maul *et al.*, 2015; Riaz *et al.*, 2018), representing an important source of diversity for future breeding programmes and sustainable agriculture (Dallakyan *et al.*, 2020). Analyses of South Caucasus grapevines by molecular markers show a moderate genetic differentiation between *V. vinifera* L. *sativa* and *sylvestris* subspecies (De Lorenzis *et al.*, 2015; Ekhvaia *et al.*, 2014; Salayeva *et al.*, 2010), high levels of genetic diversity and heterozygosity (Arroyo-García *et al.*, 2006; Imazio *et al.*, 2013), and some particular alleles, scarcely represented in other genetic pools (Riaz *et al.*, 2018). All these features are commonly found in crop domestication centers. Consistent with this evidence, archaeological and archeobotanical findings indicate that viticultural activities first began in this region during the early Neolithic Period (This *et al.*, 2006), the earliest evidence of winemaking activities in Armenia being traced back to around 4,000 BCE (Barnard *et al.*, 2011).

Nowadays, viticulture is one of the leading branches of the Armenian agricultural sector, accounting for more than 16,500 hectares of vineyards, which mainly produce grapes for wine production. According to the Vine and Wine Foundation of Armenia (https://vwfa.am/), the Armenian grape and wine sector produced 264,000 tonnes of table and wine grapes in 2020, and more than 11 M litres of wine. More than 80 % of grape production took place in the Armavir, Ararat and Aragatsotn provinces. The presence of abundant wild grapevine populations and the Armenian edafoclimatic conditions, altitudinal variation, isolated valleys and soil types likely prompted the generation of highly diverse grape varieties that are adapted to the local conditions (Dallakyan *et al.*, 2020), which have been exploited for grape cultivation and winemaking in Armenia until today (Barnard *et al.*, 2011). Nowadays, some of these varieties are exclusively found in private vineyards or gardens, where owners preserve old vines for their own consumption. Other cultivars can still be found in old viticultural areas and are at risk of extinction (Dallakyan *et al.*, 2020; Margaryan *et al.*, 2019; Nebish *et al.*, 2017). Attempts have been made to minimise the loss of these local genetic resources, including the establishment of a series of grapevine collections. The first Armenian grapevine collection was established in 1950 by the Armenian Scientific Research Institute of Viticulture, Winemaking and Fruit growing in Yerevan (FAO Institute Code: ARM 02); it held 850 accessions of local cultivars, interspecific bred hybrids, international cultivars and wild forms. After its closure in the early 1990s, many of these cultivars were irreversibly lost, and only around 140 varieties were stored in the three minor grapevine collections created at (i) the Institute of Botany of the National Academy of Sciences of Armenia in Yerevan (ARM005), (ii) the Scientific Center of Viticulture, Winemaking and Fruit growing in Yerevan (ARM 06), and (iii) the Armenian Academy of Viticulture, Wine-Making and Fruit-Growing in Nalbandyan (ARM011). In 2016, most of the genetic resources stored in the Nalbandyan grapevine collection were transferred to Echmiadzin to be part of the new Armenian Grape Collection of the Scientific Center of Agriculture (Margaryan *et al.*, 2019).

During this process, grape varieties were named under different appellations, and while new names were established in some areas, the old ones remained in others (Dallakyan *et al.*, 2020). Molecular genetic studies are useful for identifying possible synonyms (accessions with identical genetic profiles, but with different names), homonyms (accessions with identical names, but with different genetic profiles) and misnames (accessions of one cultivar registered under the name of another cultivar) in grapevine collections. Such information contributes to the true-to-type identification of local varieties, making it easier to register them in national databases and to study their genetic relationships with local and international cultivars, as has been proved in numerous studies (Carka *et al.*, 2015; Maul *et al.*, 2015; Popescu *et al.*, 2017). Nowadays, the combined use of nuclear Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP) markers has proved efficient in the genetic identification of grapevine varieties, as well as in the description of first degree genetic relationships between local and international cultivars (Cunha *et al.*, 2020; Maraš *et al.*, 2020; Zinelabidine *et al.*, 2012). When this information is combined with chloroplast genotype profiling, the maternal lineage of any cultivar can be determined, thus enabling its historical origin to be tracked down (Arroyo-García *et al.*, 2006).

TABLE 1. List of the 37 Armenian grapevine samples (AM) included in this work. When available, we indicate accession name and code, as well as berry colour (B: Black; P: Pink; R: Red; W: White), flower sex (F: Female; H: Hermaphrodite) and formation of seeds (C: Complete; NC: No Complete).

Sample ID	Accession name	Holding Institute	Accession code	Described origin	Berry colour	Flower sex	Seeds
AM01	Ararati	ARM011	V-56	Autochthonous cultivar	R	F	C
AM02	Areni	ARM011	IV-20	Autochthonous cultivar	B	H	C
AM03	Areni Clone	ARM011	IV-68	Autochthonous cultivar	B	H	C
AM04	Avagi 1	ARM011	IV-66	Unknown	B	H	C
AM05	Avagi 2	ARM011	IV-26	Unknown	B	H	C
AM06	Avagi 3	ARM011	IV-27	Unknown	B	H	C
AM07	Avagi X	ARM011	IV-28	Unknown	R	H	C
AM08	Charentsi	ARM011	VI-9	Bred cultivar	B	H	C
AM09	Eraskheni	ARM011	V-79-81	Autochthonous cultivar	B	H	C
AM10	Gervaghahas Karmir	ARM011	IV-36	Autochthonous cultivar	B	F	C
AM11	Hakobi Vordi	ARM011	IV-25	Autochthonous cultivar	R	H	C
AM12	Hastamashk	ARM011	V-47-48	Autochthonous cultivar	W	H	NC
AM13	Hayreniq	ARM011	IV-41	Bred cultivar	B	H	C
AM14	Kakhet	ARM011	V-27-28	Autochthonous cultivar	B	H	C
AM15	Kaqavik	ARM011	V-22	Autochthonous cultivar	W	H	NC
AM16	Karmir Khach	-	-	Autochthonous cultivar	R	H	C
AM17	Karmir Muscat	-	-	Autochthonous cultivar	R	F	C
AM18	Khach Kharji	ARM011	V-23	Autochthonous cultivar	W	H	C
AM19	Kharji	ARM011	V-23	Autochthonous cultivar	W	H	C
AM20	Lyustra-1	ARM011	IV-43	Unknown	B	H	C
AM21	Lyustra-2	ARM011	IV-44	Unknown	B	H	C
AM22	Mskhali	ARM011	5/27/2006	Autochthonous cultivar	W	H	C
AM23	Muscat Spitak	ARM011	9/27/2010	Autochthonous cultivar	W	H	C
AM24	Nazeli	ARM011	IV-1-25	Bred cultivar	W	H	NC
AM25	Nerkeni	ARM011	XI-8-9	Bred cultivar	B	H	C
AM26	Parvana	ARM011	28	Autochthonous cultivar	W	H	NC
AM27	Qrdi Khaghogh	ARM011	IV-70-71	Autochthonous cultivar	W	H	C
AM28	Sali	ARM011	V-32	Autochthonous cultivar	B	H	C
AM29	Sev Araqseni	ARM011	5	Autochthonous cultivar	B	H	C
AM30	Shahumyani	ARM011	V-75-76	Autochthonous cultivar	W	H	C
AM31	Tokun	ARM011	IV-30	Autochthonous cultivar	W	H	C
AM32	Tozot	ARM011	V-31	Autochthonous cultivar	B	H	C
AM33	Vaghahas Areni	ARM011	V-36	Autochthonous cultivar	B	H	C
AM34	Vani	ARM011	IV-23	Bred cultivar	W	H	C
AM35	Vanki	ARM011	IV-27	Autochthonous cultivar	P	H	C
AM36	X4	ARM011	IV-26	Unknown	W	H	C
AM37	Wild, unknown	-	-	-	-	-	-

Despite recent efforts to characterise the genetic diversity of Armenian cultivars (Dallakyan *et al.*, 2020; Margaryan *et al.*, 2019; Nebish *et al.*, 2017), local grapevine collections still hold a high number of synonyms, homonyms and misnames (Dallakyan *et al.*, 2020), which hinders their efficient conservation, characterisation, evaluation and eventual utilisation. In addition, the genetic relationships between the Armenian cultivars have been little explored, and information regarding their pedigree and likely origin is scarce. Therefore, the aim of this study was to use SSR and SNP markers in order to carry out a genetic characterisation of a series of traditional cultivars, most of them stored in the International Academy of Viticulture and Winemaking in Nalbandyan and presumed to be autochthonous Armenian varieties. From the results, it was possible to identify almost all the analysed accessions, as well as provide pedigree information about some of them, thereby improving knowledge of the Armenian grapevine genetic pool.

MATERIALS AND METHODS

1. Plant material

Thirty-seven grapevine accessions from the Grape Collection of the Armenian Academy of Viticulture, Wine-Making and Fruit-Growing in Nalbandyan, Armavir (FAO Institute Code: ARM011) and from a local private orchard (two samples: AM16 and AM17) were analysed (Table 1). Accessions in the Nalbandyan grape collection correspond to local cultivars traditionally grown in Armenia or bred cultivars from recent breeding programmes, except for one sample (AM37) which was originally collected as a feral vine. Young leaves from all accessions were collected *in situ*, dried in silica gel and stored at room temperature until DNA extraction.

2. DNA extraction and genotyping

Whole genomic DNA was extracted from approximately 100 mg of ground leaf tissue according to the methods described by Zinelabidine *et al.* (2010). DNA quality and quantity was evaluated using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA) and by visual inspection with lambda DNA on an ethidium bromide-stained agarose gel (0.8 %). All samples were then genotyped at seven SSR markers in a single multiplex polymerase chain reaction (PCR): VVMD5, VVMD7, VVMD27, VVMD32, VVS2, VrZAG62, and VrZAG79, including the reference set of six SSR markers recommended for grapevine identification (Maul *et al*., 2012; This *et al*., 2004). Multiplex PCR reaction was carried out with 5 ng of DNA, 0.07 µM of VVMD32, 0.10 µM of VrZAG62, 0.12 µM of VVS2, 0.15 µM of VVMD7 and VrZAG79, 0.35 µM of VVMD27 and 0.50 µM of VVMD5 primers using QIAGEN multiplex PCR kit (Qiagen, Hilden, Germany). The forward primer of each pair was fluorescently labelled with different dyes: 6-FAM for VVMD27 and VVMD5, VIC for VVMD32 and VrZAG62, NED for VVS2 and VVMD7, and PET for VrZAG79. Amplification reactions were performed in a Thermal Cycler T100 (Bio-Rad, Hercules, USA) using the following PCR cycle: initial denaturation at 95 °C for 15 min, followed by 30 cycles of 95 °C for 45 s, 55 °C for 60 s, and 72 °C for 30 s, and a final extension step at 72 °C for 1 h. Sample AM23 was genotyped for one additional SSR marker (VVMD28), and samples AM08 and AM25 for four additional SSRs (VrZAG112, VrZAG29, VrZAG67 and VrZAG83), following the methods detailed in Ibáñez *et al*. (2009). DNA amplification products were mixed with 20 µl of highly deionized (Hi-Di) formamide, and 0.2 µl of GeneScan-500 LIZ size standards (both from Applied Biosystems, Foster City, CA, USA) and denaturalised at 95 °C for 5 min prior to capillary electrophoresis, which was performed in the genotyping platform of the Centro de Investigación Biomédica de La Rioja (CIBIR). Fragment sizes were determined by means of GeneMapper v.4.1 (Applied Biosystems, Darmstadt, Germany). In each analysis, one Tempranillo Tinto DNA was included as a positive control.

In parallel, DNAs were profiled for a set of 240 nuclear SNP markers applying Fluidigm technology, through the genotyping services provided by the Sequencing and Genotyping Unit of the Universidad del País Vasco (UPV/EHU). This set includes a core set of 48 SNPs used for cultivar identification and an extended set of 192 SNPs for parentage and diversity analyses (Cabezas *et al.*, 2011; Cunha *et al.*, 2020; Lijavetzky *et al.*, 2007). The core set of 48 SNPs contained three chloroplast SNPs for the identification of the four most common grapevine chloroplast haplotypes (A, B, C and D) (Arroyo-García *et al.*, 2006).

3. Variety identification

Non-redundant 48-SNP genetic profiles were compared pair-wise with those stored in the ICVV-SNP database for cultivar identification.

To date, this database contains more than 3,000 unique 48-SNP genotypes. In parallel, non-redundant SSR genetic profiles were compared with those stored in the *V*IVC (Maul, 2020), the European *Vitis* Database (Maul *et al.*, 2012) and in the Armenian *Vitis* Database (Margaryan *et al.*, 2019) for cultivar identification within a broader context.

4. Phylogenetic and parentage analyses

An Unweighted Neighbor Joining (UwNJ) distance tree was calculated to explore the relationship between the Armenian cultivars considered in this work and a group of 27 traditional grapevine cultivars from ten European countries: Austria (2 cultivars), France (5), Germany (2), Italy (5), Moldova (1), Montenegro (2), Portugal (2), Serbia (1), Slovenia (2) and Spain (5) (Supplementary Table 1). The 240-SNPs genetic profiles of these 27 cultivars were retrieved from the ICVV-SNP database. For this analysis, a dissimilarity matrix with 10,000 bootstrap steps was calculated using the DARwin software package v. 6.0.21 (Perrier and Jacquemond-Collet, 2006), discarding those SNPs with any missing data (90 SNPs were removed). This matrix was used to construct an UwNJ distance tree based on 1,000 bootstrap replicates. Similarly, another UwNJ distance tree was constructed for the Armenian cultivars analysed in this study following the same procedure (81 SNPs were removed), using *V*IVC information of cultivar main use (wine/table/multi-purpose) for graphical representation.

For parentage analyses, the non-redundant 240-SNPs genotypes obtained in this study were merged with those stored in the ICVV-SNP database to detect possible first-order kinship relationships (trios and duos or parent–offspring pairs), using the likelihood-based method implemented in Cervus v.3.0 (Kalinowski *et al.*, 2007) as previously described (Cunha *et al.*, 2020). The likelihood of each detected trio and duo was evaluated taking into account the natural logarithm of the overall likelihood ratio (LOD) score. The maximum number of mismatching loci for trios and duos was set to 2 and 1 respectively, and only duos with LOD > 25 were considered. For each trio, chloroplast haplotype information was used to determine which of the putative parents acted as mother, according to the maternal transmission of chloroplasts in grapevine (Arroyo-García *et al.*, 2006).

RESULTS

The combination of SNP and SSR genotyping of the 37 accessions explored in this work revealed 27 different genetic profiles (Table 2 and Supplementary Table 2). Twenty-four grapevine cultivars were identified via the parallel comparison of the 48-SNP genetic profiles with those stored in the ICVV-SNP database, and the 7-SSR genetic profiles with international databases; the genetic profile of three accessions (AM16, AM17 and AM37), however, did not match the genetic profile of any previously registered grapevine varieties (Table 2). The set of 7-SSRs markers was informative enough for the identification of most of the accessions analysed here, except for AM23 ('Muscat Spitak'), whose genetic profile for these seven loci matched with those of 'Muscat Ottonel' (*V*IVC variety number 8243) and 'Muscat St. Laurent' (*V*IVC variety number 8252). According to the *V*IVC information, these two cultivars are full siblings from 'Chasselas Blanc' × 'Ingram's Muscat', and they differ for VVMD28 ('Muscat Ottonel': 258:268; 'Muscat St. Laurent': 218:246). After genotyping AM23 for VVMD28 by means of a simplex PCR, this accession was confidently identified as being 'Muscat Ottonel' (Supplementary Table 2), confirming the results obtained when comparing its 48-SNP genetic profile with those stored in the ICVV-SNP database, for which 'Muscat Ottonel' and 'Muscat St. Laurent' differ in 23 SNPs. Moreover, accessions AM08 ('Charentsi') and AM25 ('Nerkeni') showed the same genetic profile for the seven SSRs initially screened and for the four SSRs genotyped in an additional multiplex PCR (VrZAG112, VrZAG29, VrZAG67 and VrZAG83), but they differed in 14 of the 240 SNPs used for genetic profiling (Supplementary Table 2). For these 14 SNPs, AM25 was found to be homozygous for one of the two different alleles that were present in a heterozygous manner in AM08. Given their different SNP genetic profiles, these two accessions can be considered as two different cultivars.

As can be seen in Table 2, the vast majority of the 24 identified cultivars (19) are already registered in the *V*IVC as Armenian cultivars, whilst three are from neighbouring or Near East countries like Afghanistan ('Kandahari Siah'), Turkmenistan ('Eskeri') or Uzbekistan ('Kishmish Khishrau'), and one is from France ('Muscat Ottonel'). The most commonly found variety was 'Areni Sev', which was found six times, followed by 'Areni Spitak', 'Eskeri', 'Hakobi Vordi', 'Khusaine

TABLE 2. Results of the genetic identification of 37 grapevine accessions obtained by SNP and SSR genetic profiling.

Variety Name*	Short Name	N (Sample/s ID)	Variety number*	SNP-ICVV genotype code	Chlorotype	Use*	Origin*
Arakseni Chernyi	ACH	1 (AM29)	530	1417	D	T	Armenia
Areni Sev	ASE	6 (AM02, AM03, AM04, AM06, AM14, AM21)	576	3834	B	T/W	Armenia
Areni Spitak	ASP	2 (AM27, AM36)	577	3933	C	W	Armenia
Charentsi	CHA	1 (AM08)	2459	4009	C	W	Armenia
Eghegnadzori Sev	EGH	1 (AM05)	26229	4008	C	W	Armenia
Eraskheni	ERA	1 (AM09)	3924	4010	D	T/W	Armenia
Eskeri	ESK	2 (AM12, AM24)	3960	204	C	T/R	Turkmenistan
Hakobi Vordi	HVO	2 (AM07, AM11)	24880	3932	C	W	Armenia
Hayastan	HAY	1 (AM34)	22017	4022	C	T	Armenia
Hayreniq	HYR	1 (AM13)	16453	4012	C	T	Armenia
Kandahari Siah	KAS	1 (AM10)	5956	305	D	T	Afghanistan
Karmir Kakhani	KKA	1 (AM01)	6000	879	C	T	Armenia
Karmir Khach	KKH	1 (AM16)	-	4014	C	-	-
Karmir Muscat	KMU	1 (AM17)	-	4015	C	-	-
Khardji Sev	KHS	1 (AM20)	25976	4017	C	W	Armenia
Khatun Khardzhi	KHK	1 (AM19)	6168	4016	D	W	Armenia
Khusaine Belyi	KHB	2 (AM26, AM30)	6203	315	C	T	Armenia
Kishmish Khi-shrau	KIK	1 (AM15)	6258	4021	C	T	Uzbekistan
Masis	MAS	1 (AM22)	7470	4024	D	T	Armenia
Muscat Ottonel	MUO	1 (AM23)	8243	755	C	T/W	France
Nerkeni	NER	1 (AM25)	-	4020	C	-	-
Tokun	TOK	1 (AM31)	12558	4023	D	T/R/W	Armenia
Tozot	TOZ	2 (AM28, AM32)	12600	3823	C	W	Armenia
Unknown	UNK	1 (AM37)	-	3949	C	-	-
Vaghahas Areni	VAR	1 (AM33)	25978	3838	C	W	Armenia
Vanki	VAN	1 (AM35)	25977	4013	D	W	Armenia
Voskeat	VOS	1 (AM18)	13165	1450	D	T/W	Armenia

*According to *V*IVC database. For Use, T: Table grape; R: Raisin grape; W: Wine grape.

Belyi', and 'Tozot', all of them found twice. Regarding their main use, eight of the identified genotypes are registered in the *V*IVC database as table grape cultivars, nine as wine grape cultivars and six as cultivars with multiple uses. Chlorotype C was found to be the most abundant within the different genotypes analysed (18 times, 66.6 %), followed by chlorotype D (8 times, 29.6 %) and chlorotype B (once, 3.7 %) (Table 2).

The UwNJ distance tree constructed from the 27 unique genetic profiles identified in this work and 27 profiles from autochthonous grapevine cultivars from diverse European countries grouped the genotypes into two clusters that clearly reflected their origin (Figure 1A). The UwNJ tree constructed exclusively with the 27 unique genetic profiles identified in this study generated two main clusters (Figure 1B), which mostly reflected the main use of the cultivar, as recorded in the *V*IVC database. Thus, one cluster grouped most of the varieties with a clear aptitude for winemaking (such as the white-berried cultivar 'Areni Spitak', or the black-berried cultivars 'Khardji Sev' and 'Tozot'), whilst the other grouped most of those suitable for table grape production (such as the cultivars 'Hayastan', 'Hayreniq' and 'Masis').

Parentage analysis based on SNP data was useful for revealing the full pedigree (trio) of eight of the genotypes identified in this work (Table 3), as well as eight first-order genetic relationships (Table 4). The full pedigrees identified for 'Kishmish Khishrau', and 'Muscat Ottonel' had been already reported and confirmed in the literature (Lacombe et al., 2013), but it is the first time that the remaining six pedigrees (either previously reported in the literature or not) are

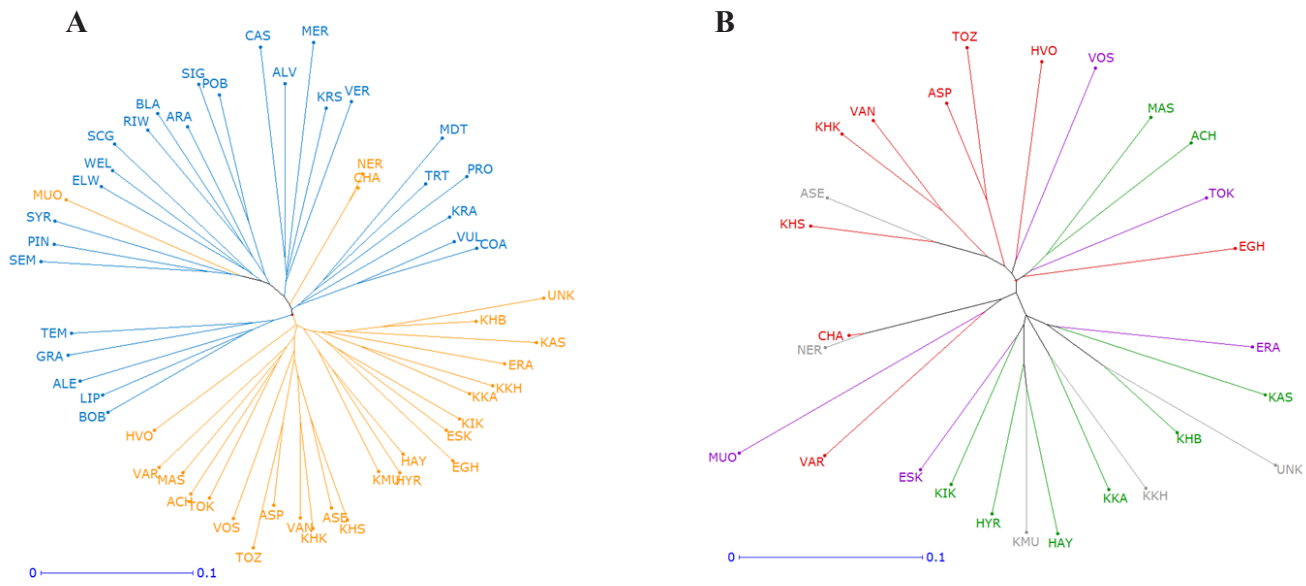

FIGURE 1. Unweighted neighbor-joining (UwNJ) unrooted trees obtained from SNP data for (a) 27 unique genotypes analysed in this work (in orange; ACH (Arakseni Chernyi), ASE (Areni Sev), ASP (Areni Spitak), CHA (Charentsi), EGH (Eghegnadzori Sev), ERA (Eraskheni), ESK (Eskeri), HVO (Hakobi Vordi), HAY (Hayastan), HYR (Hayreniq), KAS (Kandahari Siah), KKA (Karmir Kakhani), KKH (Karmir Khach), KMU (Karmir Muscat), KHS (Khardji Sev), KHK (Khatun Khardzhi), KHB (Khusaine Belyi), KIK (Kishmish Khishrau), MAS (Masis), MUO (Muscat Ottonel), NER (Nerkeni), TOK (Tokun), TOZ (Tozot), UNK (Unknown), VAR (Vaghahas Areni), VAN (Vanki), VOS (Voskeat)) and 27 genotypes for Western and Central Europe countries (in blue; ALE (Aledo), ALV (Alvarelhao), ARA (Aramon), BLA (Blaufraenkisch), BOB (Bobal), CAS (Castelao), COA (Coarna Alba), ELW (Elbling Weiss), GRA (Graciano), KRA (Kratošija), KRS (Krstac), LIP (Listán Prieto), MER (Merlot), MDT (Moscato di Terracina), PIN (Pinot), POB (Portugieser Blau), PRO (Prokupac), RIW (Riesling Weiss), SCG (Schiava Grossa), SEM (Semillon), SIG (Silvaner Gruen), SYR (Syrah), TEM (Tempranillo), TRT (Trebbiano **T**oscano), VER (Vermentino), VUL (Vulpea), WEL (Welschriesling)); and (b) 27 unique genotypes analysed in this work coloured according to their main use description available in the *V*IVC database (green: table grape; red: wine grape; purple: multi-purpose grape; grey: unknown).

TABLE 3. Summary of the full trios detected by SNP analyses involving (at least) one of the genotypes identified in this work. If available, the *V*IVC variety number is given between brackets. If not, the SNP-ICVV genotype code is provided.

Offspring*	Chlorotype	Parent 1 (mother)	Chlorotype	Parent 2 (father)	Chlorotype	SNPs compared	Mismatching SNPs	LOD score	Reference
Areni Spitak (*V*IVC: 577)	C	Tozot (*V*IVC: 12600)	C	Areni Sev (*V*IVC: 576)	B	223	0	100.3	-
Hayreniq (*V*IVC: 16453)	C	Angur Kalan (*V*IVC: 8561)	C	Alphonse Lavallee (*V*IVC: 349)	B	220	0	88.9	-
Nerkeni (ICVV: 4020)	C	Charentsi (*V*IVC: 2459)	C	Charentsi (*V*IVC: 2459)	C	201	0	83.8	-
Kishmish Khishrau (*V*IVC: 6258)	C	Angur Kalan (*V*IVC: 8561)	C	Kishmish Chernyi (*V*IVC: 6256)	D	215	0	81.6	Lacombe *et al.* (2013)
Hayastan (*V*IVC: 22017)	C	Angur Kalan (*V*IVC: 8561)	C	Italia (*V*IVC: 5582)	C	220	0	80.5	-
Muscat Ottonel (*V*IVC: 8243)	C	Ingram's Muscat (*V*IVC: 5531)	C	Chasselas Blanc (*V*IVC: 2473)	D	229	1	73.9	Laucou *et al.* (2018)
Khusaine Belyi (*V*IVC: 6203)	C	Dzhandzhal Kara (*V*IVC: 3760)	C	Unknown (ICVV: 3949)	C	223	2	70.2	-
Karmir Muscat (ICVV: 4015)	C	Angur Kalan (*V*IVC: 8561)	C	Mathiasz Janosne (*V*IVC: 7503)	B	220	0	68.2	-

*For Khusaine Belyi (*V*IVC: 6203), both parents could have acted as parent 1 (mother) or parent 2 (father).

TABLE 4. Summary of the first-order genetic relationships (duos) detected by SNP analyses involving (at least) one of the genotypes of the Armenian samples studied in this work. If available, the *V*IVC variety number is given between brackets. If not, the SNP-ICVV genetic code is provided.

Variety 1	Variety 2	SNPs compared	Mismatching SNPs	LOD score	Reference
Muscat Ottonel (*V*IVC: 8243)	Muscat St. Laurent (*V*IVC: 8252)	222	0	62.9	Lacombe *et al.* (2013)
Areni Sev (*V*IVC: 576)	Khardji Sev (*V*IVC: 25976)	221	0	48.1	-
Vanki (*V*IVC: 25977)	Khatun Khardzhi (*V*IVC: 6168)	223	0	46.3	-
Masis (*V*IVC: 7470)	Mskhali (*V*IVC: 8109)	211	0	44.4	-
Vaghahas Areni (*V*IVC: 25978)	Madeleine Angevine (*V*IVC: 7062)	218	0	37.7	-
Eskeri (*V*IVC: 3960)	Sultanina (*V*IVC: 12051)	211	0	37.6	Lacombe *et al.* (2013)
Karmir Khach (ICVV: 4014)	Karmir Kakhani (*V*IVC: 6000)	223	0	35.0	-
Areni Spitak (*V*IVC: 577)	Khardji Sev (*V*IVC: 25976)	221	1	28.2	-

supported by molecular analyses. All pedigrees were fully confirmed by means of the SSR data available, except the one proposed for 'Khusaine Belyi', which has one allele for VVS2 (147) absent in its suggested two genitors ('Khusaine Belyi' is 147:155, whereas 'Dzhandzhal Kara' and 'Unknown' (AM37) are 125:155 and 143:155 respectively) (Supplementary Table 2) (Maul, 2020). Moreover, our results indicate a relevant role for the female cultivar 'Angur Kalan' in the genotypes identified here, being a genitor in four of the eight full trios identified in this study. In addition, the genotype obtained for 'Nerkeni' was found to be compatible with a self-cross of the genotype obtained for 'Charentsi'. Lastly, six new duos were identified within the genotypes explored in this study; as shown in Table 4, in many of them the cultivars involved were Armenian, indicating the close pedigree relationships that exist among local grapevines.

DISCUSSION

Ancient and almost neglected cultivars in the South Caucasus have been found to have unique traits for facing current viticulture challenges (Bitsadze *et al.*, 2015; Sargolzaei *et al.*, 2021); this highlights the relevance of exploring local grapevine germplasm as alternative genetic sources of variability for future breeding programmes. The identification of local Armenian grapevines is the first step in their study and eventual use; however, recent reports have shown that many have not been properly identified (Dallakyan *et al.*, 2020; Margaryan *et al.*, 2019; Nebish *et al.*, 2017). In the present study, the analysis of 37 grapevine local accessions by means of SNP and SSR markers resulted in the identification of 27 unique genotypes, of which 24 were already recorded in the *V*IVC database, most of them as Armenian cultivars (Table 1). Therefore, three new genetic profiles, which were not registered in the three international databases used in the study, were identified: 'Karmir Khach', 'Karmir Muscat' and 'Unknown'. These three samples were obtained from a private orchard ('Karmir Khach' and 'Karmir Muscat', both locally appreciated for fresh fruit production), and from the wild ('Unknown'). Although the latter sample was collected in the wild, it is unlikely that it is a *sylvestris* plant, since in a preliminary genetic structure analysis between European *sativa* and *sylvestris* genotypes it clustered with the *sativa* individuals (data not shown). In addition, it was identified as one of the potential genitors of the cultivar 'Khusaine Belyi' (Table 3), so it is more likely that this 'Unknown' genotype corresponds to an ancient cultivar in traditional Armenian viticulture. The parent-offspring relationship

observed between 'Khusaine Belyi' and its other potential genitor ('Dzhandzhal Kara') has already been suggested by Lacombe *et al*. (2013) and Laucou *et al*. (2018). Because the three involved genotypes carried the chlorotype C (Table 3), it was not possible to determine which one acted as female genitor in this cross.

Parentage analyses confirmed the pedigrees previously proposed for the cultivars 'Kishmish Kishrau' ('Angur Kalan' × 'Kishmish Chernyi'), and 'Muscat Ottonel' ('Ingram's Muscat' × 'Chasselas Blanc') by means of SSR profiling (Lacombe *et al.*, 2013), and they supported the pedigree indicated for the bred cultivar 'Hayastan' ('Angur Kalan' × 'Italia'), for which no previous molecular data was available (Melyan *et al.*, 2019). In addition, our results were useful for clarifying the pedigree of the Armenian cultivar 'Hayreniq'. As declared by the breeders, 'Hayreniq' is an offspring of a cross between 'Italia' and 'Muscat Hamburg' cultivars (Golodriga *et al.*, 1984), but this pedigree is not compatible with the SSR data stored in the *V*IVC database (Maul, 2020). Our SSR and SNP results strongly indicate that 'Hayreniq' resulted from a cross between the cultivars 'Angur Kalan' and 'Alphonse Lavallee'. The female cultivar 'Angur Kalan' (syn. 'Nimrang') was found to be a major founder of local Armenian genetic resources (Table 3), which agrees with its common use as a female genitor in breeding programmes aimed at enhancing the productivity of vineyards during the development of viticulture in the South Caucasus (Maghradze *et al.*, 2020). For practical reasons, renowned breeders like Bruno Bruni, Giovanni Dalmasso, Pierre Landot or Alberto Pirovano also recurrently used female cultivars as genitors in their breeding programmes. As a result, female-flowered cultivars like 'Bicane' or 'Chaouch Blanc' figure as progenitors of numerous cultivars bred during the nineteenth and the beginning of the twentieth centuries, especially of table grapes (Lacombe *et al*., 2013; Vargas *et al*., 2009). Furthermore, the results of the present study, indicate that 'Nerkeni' (AM25) may be the result of the self-pollination of 'Charentsi' (AM08). Although hermaphrodite *V. vinifera* genotypes can theoretically be selfed, this is not a common mechanism for creating new cultivars in *V. vinifera*, and only few examples of cultivated varieties derived from self-pollination events have been molecularly confirmed (such as 'Covè' or 'Čubrica-2', selfings of 'Harslevelu' and 'Čubrica' respectively (Cipriani *et al.*, 2010; Maraš *et al.*, 2020)). In fact, selfed offsprings are at a disadvantage, because inbreeding exposes the harmful nature of deleterious mutations hidden in heterozygous state (Zhou *et al*., 2019). Interestingly, the *V*IVC database includes a variety named 'Nerkeni' (*V*IVC variety code: 8489), but unfortunately SSR information is lacking for a direct comparison to be made with the genetic profile obtained in this study. Nevertheless, the pedigree indicated by the breeders for this variety ('Saperavi' × Mixture of pollen Areni (Malan) + Kachet + Hybrid Nº24 (= Garandmak x Richter 31)) (Katarjan, 1962) is not compatible with the genetic profile obtained for 'Nerkeni'; for example, the proposed female genitor of 'Nerkeni' ('Saperavi') is 239:239 and 188:200 for VVMD7 and VrZAG62 respectively (Maul, 2020), whilst the profile obtained for 'Nerkeni' (AM25) was 247:249 and 194:196 for the same two loci (Supplementary Table 2). This suggests that (a) the pedigree data is wrong (as also observed for 'Hayreniq'), or (b) it is one case of homonymy, and the 'Nerkeni' studied here corresponds to a different cultivar with the same name.

In the present study, multiple cases of synonyms, homonyms and misnames were revealed. As an example of synonymy, up to six different accessions registered under diverse local names were genetically identified as 'Areni Sev' (Table 2). In some cases, these synonyms indicate some particular phenotypic features of the accession, like the one observed for the 'Areni Sev' accession registered as 'Lyustra-2'. Lyustra means 'chandelier' in Russian, which reflects the singular loose cluster architecture of this 'Areni Sev' accession. The high prevalence of 'Areni Sev' within the set of accessions analysed in this work agrees with the high relevance of this cultivar for Armenian viticulture, which has been widely used for red wine elaboration in different Armenian regions for centuries (Dallakyan *et al.*, 2020). Other cases of synonymy were detected for the cultivars 'Arakseni Chernyi' (AM29), 'Kishmish Khishrau' (AM15), 'Tozot' (AM32), and 'Voskeat' (AM18), which were registered under the local names 'Sev Araqseni', 'Kaqavik', 'Sali', and 'Kharch Kharji' respectively. Interestingly, the cultivar 'Muscat Ottonel' was found in the grape collection under the local name 'Muscat Spitak' (AM23), despite this term being registered as a synonym for the cultivar 'Muscat a Petits Grains Blancs' (Maul, 2020). Regarding the detected homonyms, accessions registered as 'Avagi' were identified as 'Areni Sev', 'Eghegnadzori sev' and 'Hakobi Vordi', and accessions named 'Lyustra' were identified as 'Areni Sev' and 'Khardji sev'.

On the other hand, two accessions (AM26 and AM30, 'Parvana' and 'Shahumyani' respectively) were identified as 'Khusaine Belyi', a white-berried table cultivar existing in numerous grapevine collections under many synonyms (Maul, 2020) and progenitor of other table grape cultivars (Lacombe *et al.*, 2013). Accession AM30 is seeded (Table 1), which agrees with the seed phenotype recorded for 'Khusaine Belyi' in the *V*IVC database (Maul, 2020). Interestingly, accession AM26 has been previously described as stenospermocarpic (Nebish *et al.*, 2015), suggesting that it could be a 'Khusaine Belyi' seedless somatic variant. If confirmed, the analysis of these two accessions could be of high interest for determining the genetic and molecular causes of this seedless phenotype, and thus providing new insights into this relevant trait for table grape breeding, as recently done with other variant pairs (Costantini *et al.*, 2021; Royo *et al.*, 2016; Royo *et al.*, 2018). The seedless accession AM26 was found to be wrongly named 'Parvana', which is another Armenian white-berried seedless cultivar with a similar cluster phenotype to that of AM26, what could be the origin of the misnaming. Unfortunately, this was not the only misnamed accession, as the accessions wrongly named under the cultivar names 'Ararati' (AM01), 'Kakhet' (AM14), 'Mskhali' (AM22), 'Qrdi Khaghogh' (AM27), and 'Vani' (AM34), were found to correspond to the cultivars 'Karmir Kakhani', 'Areni Sev', 'Masis', 'Areni Spitak' and 'Hayastan' respectively (Table 2).

Lastly, the studied genotypes showed a clear genetic differentiation from the set of Western and Central European varieties used as a reference (Figure 1A), and they had some microsatellite alleles poorly represented in other genetic pools (like the 292 allele detected in VVMD32) – which are two of the expected features of a putative domestication centre (Sargolzaei *et al.*, 2021). These results are in line with the generally accepted assumption of the South Caucasus as the cradle of grapevine domestication, which has been widely supported by genetic, archaeological and cultural evidence (Arroyo-García *et al.*, 2006; Bouby *et al.*, 2021; McGovern *et al.*, 1997; Riaz *et al.*, 2018). In addition, we found a predominance of chlorotypes C and D in the identified genotypes, which is commonly observed among cultivars from Near East and Middle East regions (Arroyo-García *et al.*, 2006).

CONCLUSIONS

Our results help better understand the diversity of the Armenian grapevine germplasm, providing useful information for its correct identification. Despite the reduced number of accessions analysed, several cases of synonyms, homonyms and misnames were revealed. The pedigrees of several Armenian varieties have been confirmed and some others proposed for the first time. Other additional compatible parent-offspring relationships have been found, which need to be confirmed in future analyses. Furthermore, the potential finding of a novel seedless somatic variant highlights the importance of exploring the Armenian genetic pool, in order to study and exploit novel sources of genetic diversity for breeding novel cultivars which meet consumer expectations and current viticulture challenges. Unfortunately, the Nalbandyan Grape Collection of the Armenian Academy of Viticulture, Wine-Making and Fruit-Growing is no longer operational, but the majority of the accessions analysed in this study have been transferred to the Armenian National Grape Collection of the Scientific Center of Agriculture in Echmiadzin (Armavir, Armenia). Thus, the information provided in this study will be useful for curating and updating information about the grapevine germplasm preserved in this new collection.

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