

RESEARCH ARTICLE

(Open Access)**Ampelographic Characteristics and Molecular Investigation of Azerbaijani Local Grape Varieties by Microsatellites**VUGAR SALIMOV¹, GABRIELLA DE LORENZIS², RAUF ASADULLAYEV^{3*}^{1,3}Scientific Research Institute of Viticulture and Winemaking, AZ 0118, Mehdiabad settl., Baku, Azerbaijan²Dipartimento di Scienze Agrarie ed Ambientali, Università degli Studi di Milano, Via Celoria 2 - 20133, Milano, Italy**Abstract**

The article aims at introducing of some local grapevine varieties cultivated in different areas of Azerbaijan. The cultivars are grown in the ampelographic collection of the Azerbaijani Scientific Research Institute of Viticulture and Wine-making. The description of their ampelographic specifications was based on the OIV list. Comparing cultivars, it has been found that, although there are similarities in various descriptor traits, most of the characteristics are distinctive of specific cultivars. A considerable polymorphism was found concerning the botanical features of leaves, clusters, berries, as well as considering some biological and technological characteristics. In more details, these genotypes differ each other by the morphological features of leaves; the shape, size and structure of clusters; the shape, color and flavor of berries; the productivity indices; the resistance to pests and diseases; the duration of their vegetative period; the sugar and acid contents; and the number of seeds in the berry.

This study was carried out on nine grape cultivars grown in region Garabagh, Nakhchyvan and Absheron in 2008-2012. Analyses of the ampelographic features of the studied grape varieties showed that the genotypes considerably differ by their main morphological, biological and technological characteristics.

42 Azerbaijani grape varieties (including above mentioned) have been investigated comparatively with Caucasian countries` and European grape varieties. Based on the descriptive statistics Azerbaijani samples appeared having the highest genetic diversity. The first two coordinates of the PCoA differentiated the samples into two main clusters, despite the presence of overlapping zones: i) the group clusterizing the most part of cultivars from Azerbaijan; ii) the other Caucasian and European varieties. STRUCTURE analysis revealed the optimal K value equal to 3, highlighting 3 groups: i) Central European group; ii) an admixed group, containing Armenian, Georgian and Moldavian samples; iii) the group of Azerbaijani cultivars (94%) . en investigated comparatively with Caucasian countries` and European grape varieties.

Keywords: grape, variety, bunch, berry, ampelographic, collection.

1. Introduction

In Azerbaijan, a big number of wild grapevines (*Vitis vinifera* L. subsp. *sylvestris*) and local cultivars can be observed. Archeological, paleobotanical and historical sources confirm that grapevines were spread and cultivated since a long time. One-two million years old grape leave fossilized imprints have been found in the western sides of the Bozdag mountain (Gyok-gol region) and 500 000 years old grapevine fossils were found in Nakhichevan, in the Araz river basin. Grape seed remains of the V – IV century B.C. were also found in the Agstafa region. One of the most ancient wine-making artifacts in Azerbaijan is a wine jug found by Hummel in 1931 in the Gyok-gol region. It contains grape seeds and fossilized wine sediments. This finding is related to the Bronze Age

(end of II and beginning of I millennium B.C.) [1, 6, 13, 22, 23, 24].

In the period of intensive development of the viticulture in Azerbaijan (1970-80s), the grapevine assortment of vineyards was enriched with foreign varieties from West Europe, America, Moldova, Ukraine, Georgia and Central Asia. According to the literature, in that period more than 600 grapevine varieties were cultivated in Azerbaijan and 400 of them were local ones. However, only 200 of them have been collected and included in field collections. [18, 19, 20, 21]. Many regions of Azerbaijan are rich in valuable local grapevine varieties which have not been explored yet [14, 15, 22].

Nowadays, Azerbaijan local grape germplasm cultivars are studied with the support of local and

*Corresponding author: Rauf Asadullayev; E-mail: raufasad@mail.ru

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foreign scientific organizations. Thanks to Bioversity International and the COST Action FA1003 (East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding), researches on local grapes are carried out in Azerbaijan. Scientific expeditions have been conducted in different Azerbaijan regions (Garabagh, Quba-Khachmaz, Ganja-Gazakh, Nakhichevan, Sheki-Zaqatala, Shirvan, Apsheron etc.). The main obtained results include: the finding of some unstudied local genotypes; their collection in experimental vineyards; and their ampelographic evaluation. Hereby we give the description of ampelographic specifications of some valuable grape varieties cultivated in the ampelographic collection of Azerbaijani Scientific Research Institute of Viticulture and Wine-making using the OIV ampelodescriptors. The genetic diversity of Caucasian grapevine germplasm (wild and cultivated), considered the first centre of domestication, was investigated by molecular and ampelographic analysis [2, 7, 10, 12].

The *S.Myles et al.* [12] work revealed a Near East origin of *V. vinifera* and an East-to-West dissemination gradient, following the grapevine spread routes. The results reported in R. Bacilieri *et al.* [2] highlighted the genetic identity and the originality of Georgian germplasm in respect to the worldwide accessions, as well as the data described by other authors [7, 10]. The Simple Sequence Repeat (SSR) or microsatellite markers are the most used tools to analyze the worldwide grapevine germplasm, as documented by different works describing the genetic characterization of the most important grapevine repositories [8]. Microsatellites are largely used for genotyping, to solve cases of homonyms and synonyms, to determine genetic diversity of *V. vinifera* cultivars [2, 3, 10] and to establish pedigree analysis [11]. In order to investigate the genetic relationships among samples along the East-to-West dissemination route of grapevine, the aim of this work was to study the genetic diversity of germplasm coming from Armenia, Azerbaijan, Georgia and Moldova by 10 SSR markers.

2. Material and Methods

Plants of the local grape varieties 'Ikijinsli Ag Shany', 'Khurmayi', 'Gilamy', 'Ag meleyi', 'Sabza', 'Gyrmyzy saabi', 'Novrast', 'Ag Khalily', 'Shahani', 'Ag Darbandy', 'Mahmudu' and 'Khan uzumu', were all grown in the same ampelographic collection of

Azerbaijani Scientific Research Institute of Viticulture and Wine-making.

Morphological, biological and technological characterizations were coded according to 47 OIV descriptors [4, 25].

Plant materials: Autochthonous grapevine cultivars coming from Azerbaijan (42), Georgia (44), Moldova (23) and Armenia (29) randomly selected among the local germplasm of each Country, were taken into account for this study. Twenty-two European varieties were included as outgroup. The samples were sent by cuttings to the Dip. di Scienze Agrarie e Ambientali (Milano), planted in pots and grown in greenhouse.

DNA extraction: Extraction of genomic DNA was performed per each sample using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacture's instructions. Dried young leaves (0.02 g) were ground by liquid nitrogen and the powder was used to perform the DNA extraction.

SSR amplification and detection: The samples were genotyped by 10 SSR markers: VrZag62; VrZag79; VVMD5; VVMD7; VVMD27; VVMD28; VVMD21; VVMD24; VVMD25; VVS2 (LAUCOU *et al.* 2011). Multiplexed PCR amplifications were performed in 25 µl final volume reaction mixture following the method described in De Lorenzis *et al.* [5]. The PCR products were carried out on ABI PRISM 310 Genetic Analyser (Applied Biosystems by Life Technologies, Foster City, USA) and the alleles were sized by GENEMAPPER 4.0 (Applied Biosystems by Life Technologies).

Data analysis: In order to estimate the genetic diversity the different germplasms, the SSR data were used to determine the number of different alleles (Na), the effective number of alleles (Ne), the observed (Ho) and expected heterozygosity (He) per each germplasm. These data were performed by GenAlEx 6.5 software [16].

The structure and the association among the different germplasm were investigated following two different approach: i) Principal Coordinate Analysis (PCoA) [17] used to capture the correlation between genotypes; ii) STRUCTURE analysis, 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 6.5 software, starting to the SSR correlation matrix. The STRUCTURE analysis was carried out using STRUCTURE software package. K value (K number of ancestral genetic

groups) was chosen according to G.Evanno *et al.* [9]. The genetic distance and inbreeding among the population were performed considering pairwise Nei's genetic distance and pairwise F_{st} analysis. The parameters were carried out in GenAIEx 6.5 software.

3. Results and Discussion

In Azerbaijan, viticulture and wine-making developed during centuries, producing hundreds of grape varieties selected for different purposes (table, wine, universal, seedless raisins) [18, 19, 20, 21].

Local grape varieties are mainly grown in old vineyards located in ancient settlements and homesteads. Different training systems were also found, including suspended and creeping cultivations.. The varieties presented in this article ('Ikijinsli Ag shany', 'Khurmayi', 'Gilami', 'Ag meleyi', 'Sabza', 'Qyrmyzy saabi', 'Novrast', 'Ag Khalily', 'Shahani', 'Ag Darbandy', 'Mahmudu', 'Khan uzumu') differ concerning their morphological characteristics and sizes of the bunches and berries; vegetative phenology; ripening time; productivity and quality indices.

The cultivars have been characterized by 47 OIV (2009) ampelodescriptors. Results show that, although there are several similarities in the descriptor traits of some grape varieties, most characteristics considerably differ between cultivars. It was found that all the studied varieties are similar in Young shoot's opening type (OIV 001); Number of consecutive tendrils of the shoot (OIV 016); Area of anthocyanin coloration of main veins on upper side of blade of the mature leaf (OIV 070) (except 'Mahmudu'); Goffering of blade of the mature leaf (OIV 072); Teeth in the petiole sinus of mature leaf (OIV 81-1) (except 'Ag Darbandy'); Petiole sinus base limited by vein of mature leaf (OIV 081-2); Teeth in the upper lateral sinuses of mature leaf (OIV 083-2); Density of erect hairs on main veins on lower side of blade of mature leaf (OIV 087) (except 'Ag Darbandy'); Fertility of basal buds (buds 1-3) of the shoot (OIV 155) (except 'Khan uzumu'); Shape of the bunch (OIV 208); Number of wings of the primary bunch (OIV 209) (except 'Shahani'); Intensity of flesh anthocyanin coloration of the berry (OIV 231); Particular flavor of the berry (OIV 236) (except 'Mahmudu'); Formation of seeds of the berry (OIV

241) (except 'Sabza'); Time of bud burst (OIV 301) (except 'Ag Khalily'); Total acidity of must (OIV 506). Concerning the other studied descriptors, the varieties considerably differ. The size of bunches and berries, the plant productivity and the grape quality of the studied cultivars are the typical ones for the table grape varieties. As an example, the length of the berries (OIV 220) are distinguished by large and very large sizes. One of the studied varieties ('Sabza') is seedless, however the berry length is much higher than the one expected in raisin grape cultivars. The varieties also differ for berry shapes. Productivity and sugar contents of all the varieties (except 'Ag meleyi') are high and very high. The cultivars are characterized by a high vigor.

The studied genotypes differ from each other by the morphological features of leaves; the shape, size and structure of clusters; the shape, color and flavor of berries; the productivity indices; the resistance to the pests and diseases; the phenological characteristics; the sugar and acid contents; and the number of seeds per berry. (Table 1).

Biomorphological and technological characteristics of the local and introduced grape varieties collected in the Azerbaijani Scientific Research Institute of Viticulture and Wine-making have been studied and presented in this paper. The ampelographic descriptions will be useful to select material for specific purposes. The most suitable training systems could also be recommended for production based on cultivars characteristics.

Although the studied grape varieties have several similarities in morphological characteristics, it doesn't mean they belong to the same variety. So, study of the grape varieties with the studied ampelographic characteristics, using the modern molecular-genetic methods, are important in respect to the characterization of the differences of the studied varieties. With this purpose in the frames of "East-West collaboration for grapevine diversity exploration and mobilization of adaptive traits for breeding" (COST Action FA1003) project some molecular-genetic characteristics of 42 Azerbaijani grape varieties (including above mentioned) have been investigated comparatively with Caucasian countries' and European grape varieties.

Table 1. Ampelographic characteristic of Azerbaijani grape cultivars were determined according to Descriptors of Grape norms of OIV

OIV desc- riptsors	VARIETY NAME											
	Ikjinsli Ag shany	Khurma yi	Gilami	Ag meleyi	Sabza	Gyrmyzy saaby	Novrast	Ag Khalily	Shahani	Ag Darbandy	Mahmudu	Khan uzumu
1	2	3	4	5	6	7	8	9	10	11	12	13
001	5-fully open	5-fully open	5-fully open	5-fully open	5-fully open	5-fully open	5-fully open	5-fully open	5-fully open	5-fully open	5-fully open	5-fully open
003	1- none or very low	3-low	3-low	3-low	3-low	3-low	5- medium	1- none or very low	3-low	5-medium	3-low	3-low
004	3-low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	3-low	3-low	5-medium	1- none or very low
006	5- horizontal	3- semi- erect	3- semi-erect	3- semi- erect	3- semi-erect	3- semi-erect	3- semi-erect	3- semi-erect	5- horizontal	5- horizontal	3- semi-erect or 5- horizontal	3- semi-erect
007	1- green	1- green	2- green & red	1- green	1- green	1- green	2- green & red	1- green	1- green	1- green	2- green and red	2- green and red
008	1- green	1- green	2- green & red	1- green	1- green	1- green	2- green & red	1- green	1- green	1- green	2- green and red	2- green and red
016	1- 2 or less	1- 2 or less	1- 2 or less	1- 2 or less	1- 2 or less	1- 2 or less	1- 2 or less	1- 2 or less	1- 2 or less	1- 2 or less	1- 2 or less	1- 2 or less
051	2- yellow	1- green	3-bronze	3-bronze	3-bronze	3-bronze	4-copper- reddish	2- yellow or 3- bronze	2- yellow	3-bronze	2- yellow or 3- bronze	1- green
053	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	7- high	1- none or very low
067	4- circular	4- circular	4- circular	4- circular	3- pentagonal	4- circular	3- pentagonal	3- pentagonal	4- circular	3- pentagonal	4- circular	4- circular
068	2- three	2- three	3- five	3- five	3- five	3- five	3- five and 4- seven	2- three or 3- five	2- three or 3- five	3- five	2- three or 3- five	1- one (entire leaf) or 2- three
070	1- only at the petiolar point	1- only at the petiolar point	1- only at the petiolar point	1- only at the petiolar point	1- only at the petiolar point	2- only at the petiolar point	1- only at the petiolar point					
072	1- absent or very weak	1- absent or very weak	1- absent or very weak	1- absent or very weak	1- absent or very weak	1- absent or very weak	1- absent or very weak	1- absent or very weak	1- absent or very weak	1- absent or very weak	1- absent or very weak	1- absent or very weak
074	1-flat	1-flat	2- V-shaped	1-flat	2- V-shaped	2- V-shaped	2- V-shaped	1-flat	1-flat	5- twisted	1-flat	1-flat
075	1- absent or very weak	1- absent or very weak	3-weark	3-weark	1- absent or very weak	1- absent or very weak	3-weark	1- absent or very weak	1- absent or very weak	1- absent or very weak	5- medium	1- absent or very weak
076	3- both sides convex	2- both sides straight	3- both sides convex	3- both sides convex	3- both sides convex	3- both sides convex	3- both sides convex	2- both sides straight	3- both sides convex	3- both sides convex	3- both sides convex	2- both sides straight
079	9- strongly overlapped	3- half open	3- half open	3- half open	3- half open	3- half open	3- half open	7- overlapped	3- half open	9- strongly overlapped	3- half open or 5 - closed	3- half open
080	1- U-shaped	3- V-shaped	2- brace- shaped ({})	3- V-shaped	3- V-shaped	3- V-shaped	3- V-shaped	1- U-shaped	1- U-shaped	1- U-shaped	2- brace-shaped ({})	2- brace-shaped ({}) or 3-Vshaped
081-1	1- absent	1- absent	1- absent	1- absent	1- absent	1- absent	1- absent	1- absent	1- absent	9-present	1- absent	1- absent
081-2	1- not limited	1- not limited	1- not limited	1- not limited	1- not limited	1- not limited	1- not limited	1- not limited	1- not limited	1- not limited	1- not limited	1- not limited
083-2	1- absent	1- absent	1- absent	1- absent	1- absent	1- absent	1- absent	1- absent	1- absent	1- absent	1- absent	1- absent
084	1- none or very low	3- low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	7- high	3- low
087	1- none or very low	1- none or very low	3- low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	1- none or very low	3- low	1- none or very low	1- none or very low
094	1- absent or very shallow	1- absent or very shallow	7-deep	7-deep	5- medium	5- medium	7-deep	5- medium or 7- deep	3-shallow	5- medium or 7- deep	1- absent or very shallow or 3- shallow	1- absent or very shallow

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151	3- fully developed stamens and fully developed gynoecium	3- fully developed stamens and fully developed gynoecium	4- reflexed stamens and fully developed gynoecium	3- fully developed stamens and fully developed gynoecium	3- fully developed stamens and fully developed gynoecium	4- reflexed stamens and fully developed gynoecium	3- fully developed stamens and fully developed gynoecium	3- fully developed stamens and fully developed gynoecium	4- reflexed stamens and fully developed gynoecium	3- fully developed stamens and fully developed gynoecium	3- fully developed stamens and fully developed gynoecium	3- fully developed stamens and fully developed gynoecium
155	1- very low	5-medium	1- very low	1- very low	1- very low	1- very low	1- very low	1- very low	1- very low	1- very low	1- very low	5-medium
202	9-very long	9-very long	7-long	7- long	5- medium or 7-long	7- long or 9-very long	9-very long	5- medium or 7-long	5- medium or 7-long	5- medium or 7-long	7- long or 9-very long	9-very long
204	5- medium	5- medium	5- medium	3- loose	9- very dense	5- medium	5- medium	5- medium	1- very loose	7- dense	3- loose	5- medium
206	3-short	3-short	3-short	3-short	3-short	3-short	5- medium	3-short	7- long	3-short	3-short or 5- med.	3-short
208	2- conical	2- conical	2- conical	2- conical	2- conical	2- conical	2- conical	2- conical	2- conical or 3- funnel shaped	2- conical	2- conical	2- conical
209	2- (1-2 wings)	2- (1-2 wings)	2- (1-2 wings)	2- (1-2 wings)	2- (1-2 wings)	2- (1-2 wings)	2- (1-2 wings)	2- (1-2 wings)	1- absent	2- (1-2 wings)	2- (1-2 wings) or 3- (3-4 wings)	2- (1-2 wings)
220	7- long	7- long	5- medium or 7- long	5- medium or 7- long	3- short	9- very long	9- very long	7- long	7- long	9- very long	7- long or 9- very long	7- long or 9- very long
221	7- wide or 9-very wide	7- wide or 9-very wide	7- wide	7- wide	3-narrow	7- wide or 9-very wide	7- wide or 9-very wide	7- wide	7- wide	7- wide or 9-very wide	7- wide	7- wide or 9-very wide
223	7- ovoid	7- ovoid	3- broad ellipsoid	3- broad ellipsoid	7- ovoid	5- cylindrical	8- obovoid	5- cylindrical	6- obtuse ovoid	7- ovoid	2- globose	7- ovoid
225	1- green yellow	6- blue black	6- blue black	1- green yellow	1- green yellow	3- red	1- green yellow	1- green yellow	1- green yellow	1- green yellow	6- blue black	6- blue black
231	1- none or very weak	1- none or very weak	1- none or very weak	1- none or very weak	1- none or very weak	1- none or very weak	1- none or very weak	1- none or very weak	1- none or very weak	1- none or very weak	1- none or very weak	1- none or very weak
235	2- slightly firm	3- very firm	1- soft	2- slightly firm	2- slightly firm	2- slightly firm	2- slightly firm	1- soft or 2- slightly firm	2- slightly firm	2- slightly firm	2- slightly firm or 3- very firm	3- very firm
236	1-none	1-none	1-none	1-none	1-none	1-none	1-none	1-none	1-none	1-none	5- other flavor than muscat, foxy or herbaceous	1-none
241	3- complete	3- complete	3- complete	3- complete	2- rudimentary	3- complete	3- complete	3- complete	3- complete	3- complete	3- complete	3- complete
301	5- medium	5- medium	5- medium	5- medium	5- medium	5- medium	5- medium	3- early	5- medium	5- medium	5- medium	5- medium
303	5- medium	3- early	5- medium	5- medium	3- early	5- medium	3- early	1- very early	5- medium	7- late	5- medium	3- early
351	7- strong	7- strong	7- strong	5- medium	7- strong	7- strong	9- very strong	7- strong	7- strong	9- very strong	7- strong or 9- very strong	7- strong
502	7- high	7- high	7- high	3- low or 5- medium	7- high	7- high	7- high	3- low	7- high	7- high	7- high	7- high or 9- very high
503	7- high	7- high	5- medium	5- medium	3- low	7- high or 9- very high	7- high or 9- very high	5- medium or 7- high	5- medium or 7- high	7- high	7- high	7- high
504	9- very high	9- very high	7- high	5- medium	9- very high	9- very high	9- very high	5- medium	5- medium	9- very high	9- very high	9- very high
505	9- very high	9- very high	7- high	9- very high	7- high	7- high	9- very high	7- high	9- very high	7- high	9- very high	9- very high
506	3- low	3- low	3- low	3- low	3- low	3- low	3- low	3- low	3- low	3- low	3- low	3- low

The Azerbaijan republic with regard to assortment of a grapes is on a joint of two influences. As it known, assortment a grapes of Georgia (especially of western part) includes forms that belong to ecological-geographical group of the Black sea basin (*convar pontica* Negr.). In assortment of Central Asia`s republics prevail naked-leafed forms which belong to east ecological-geographical group (*convar orientalis* Negr.). On assortment of Azerbaijan`s grape varieties the strongest influence was rendered by east group (80 % of all varieties). The local grape assortment in Nakhichevan region for 85 % consists of east group varieties, from which 65 % are table varieties (Shafeiyi, Bendi, Nagshabi, etc.), 20 % are wine varieties (Malagi, Gara Aldara, Gara Sarma, etc.) and 15 % are the Black sea basin varieties (Ag Kalambir, etc.). There are East group and the Black sea basin varieties in the Absheron`s grape assortment. A number of these varieties such as Ag Gyoibendam, Gara Pishras, Gara Shany, etc. has the attributes of east group varieties (large size of bunches and berries) and of the Black sea basin varieties (felt-fluffy leaves). It is certainly possible to speak about a hybrid nature of these varieties.

The people of Azerbaijan has the ancient viticulture and wine-making traditions. Historically our people cultivated the grape for fresh consumption, to prepare the products like raisins, jam, vinegar, sherbet, wine, spirit etc., and for decorative and ornamental purposes. As we can see, our people developed viticulture and wine-making not blindly, but purposefully, and the folk selection gifted to Azerbaijani biodiversity the hundreds of valuable grape varieties of different use directions (table, wine, universal, seedless raisins) [18, 19, 20, 21].

Our people named the grape varieties by their color (Ag shany – *White shany*, Gara shany – *Black shany*, Sarygila – *Yellow berry*, Qyrmyzy saaby – *Red saaby*, Qyzyl uzum – *Red grape*, Banovshayi uzum – *Violet grape*, Goy gezendayi – *Blue gezendayi*, Chil uzum – *Multicolored grape*), quality, taste, aroma (Gulabi – *smelling like the rose water*, Shekeri – *sweet like the sugar*, Shireyi – *Juicy*, Karimqandi – *Karim`s sugar*, Qara gand uzum – *Black sugar grape*), the shape and size of the berries (Hachabash – *Doubled head*, Qush ureyi – *Bird heart*, Baba beli – *Dad`s back*, Tulku quyruğu – *Fox tail*, Deve gyozu – *Camel eye*, Tula gyozu – *Dog eye*, Kechimemesi – *Goat dug*, Inek emdjeyi – *Cow dug*, Gelinbarmagy – *Bride`s finger*, Khatynbarmagy – *Lady`s finger*, Gyozel uzum

– *Nice grape*, Iri salkhym – *Large bunch*, Sykh salkhym – *Dense bunch* etc.), ripening time (Nubari – *First yield*, Aga gyormez – *“the owner would not see”*, Kal uzum – *Unripe grape*, Gej deyen – *Late ripening* etc.), thickness of the peel and the consistence of the pulp (Khart-khart – *Crispy*, Dash uzum – *Stone grape*, Qalyn qabyq – *Thick peel*, Nazik qabyq – *Thin peel*, Dash kishmish – *Stone raisin* etc.), size of the seeds and berries and the look of the bunch (Sapda durmaz – *Would not stay at the stem*, Misqaly – *1 misqal (about 4,26 grammes) in weight*, Bandi, Tabarza etc., by medical importance and direction of use (Shafeyi – *Healing*, Sirkeyi – *Vinegar*, Sharaby – *Wine*, Kishmish, Movuju – *Raisin*, Shekerbura – *Bisquit* etc.), by spread and cultivation area (Tabrizi, Beylagani, Bayanshira, Madrasa, Shirvanshahy, Ordubadi, Shahtakhty, Shabrany, Darbandy, Aldera, Khazri etc.). some varieties are named in honour of the persons that bred or cultivated them (Khalily, Askeri, Huseyni, Talibi, Yaqubi, Zeynebi etc.).

Naturally, the grape variety is the basis for developing the viticulture. During the evolution period our people bred hundreds of valuable grape varieties. It`s gratifying to know that the most productive and high quality grape varieties, including the valuable seedless grapes (Bayanshira, Shirvanshahy, Madrasa, Tabrizi, Huseyni, Hamashara, Gyrmyzy saaby, Ag kishmish, Ag oval kishmish, Chehrayi kishmish, Gyrmyzy kishmish, Novrast, Ag Khalily, Shabrany, Shamakhy marandisy etc.) grow in our country. So, a plant of Bayanshira variety depending the shaping gives 15 to 35 kgs of fruit and more. The berries of Shirvanshahy variety collect up to 27-33% of sugar in the conditions of Kurdamir region.

It was found during the scientific expeditions that the local grape varieties are cultivated mainly in ancient settlements in old vineyards, homestead lands by different growing methods (suspended or creeping formations). The varieties presented in the article (*Ikijinsli Ag shany*, *Khurmayi*, *Gilami*, *Ag meleyi*, *Sabza*, *Qyrmyzy saabi*, *Novrast*, *Ag Khalily*, *Shahani*, *Ag Darbandy*, *Mahmudu*, *Khan uzumu*) differ by the morphological specifications and sizes of the bunches and berries, vegetation period, ripening time, productivity and quality indices.

Ikijinsli ag shany is one of the ancient table grape varieties of Apsheron region. Widely spread in the most villages and settlements of Apsheron peninsula (Mardakan, Shuvelan, Turkan, Mehdiabad,

Shagan etc.). The variety is high productive, high quality and of salable condition. By its morphological and biological specifications Ikijsli ag shany is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.) Ikijsli ag shany is considered as a variation of Ag shany variety which has the functionally female type of flower. Used mainly fresh. The bunches and berries are large with the beautiful appearance. Fully ripened berries are golden or amber colored. The surface is pruinose.

The homeland for *Khurmayi* variety are considered Nakhichevan and Apsheron regions of Azerbaijan. The best plants are found in Apsheron region. Relatively early maturing variety. In conditions of Apsheron fully ripens in late July – mid August. As an early maturing variety is in great demand among the local population. By its morphological and biological specifications *Khurmayi* is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.) The bunches and berries are large with the beautiful appearance. The berries have the specific flavour and taste. Despite being the early ripening variety, can stay on the plant for long period. The plants are strong and rapidly growing. Used fresh and for preparing the raisin.

Gilami is one of the most ancient varieties of Apsheron. The plants of this variety are found in Mashtaga, Shuvelan, Buzovna, Shagan and other villages of Apsheron. By its morphological and biological specifications *Gilami* is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.). The bunches and berries have the beautiful appearance, the berries have specific nice flavor. Although crispy, berries' pulp is juicy enough. Suitable for long term storage and transportation. Used fresh and for preparing of juice, doshab (grape honey) etc. Ripening period – mid and late August.

Ag meleyi is one of the ancient universal varieties of Nakhichevan. The plants of the variety are found in the old vineyards of Nakhichevan. By its morphological and biological specifications *Ag meleyi* is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.) The bunches' and berries' size is average or large. Berries' pulp is consistent and juicy. The peel is of average thickness and durability. The surface is pruinose. The bunches and berries have the beautiful appearance. Used fresh and for preparing of juice,

doshab (grape honey) and white dry wine. Ripening period from late August to early September.

Sabza variety is found on the old vineyards in the ancient settlements of Apsheron (Buzovna, Turkan, Novkhany, Fatmai). By its morphological and biological specifications *Ag meleyi* is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.) The seedless variety. The bunches and berries have the beautiful appearance. The berries have harmonic and nice flavor. Ripening period – mid and late August. By their bunches and berries resemble Azerbaijani famous and wide spread variety *Ag oval kishmish*. On the surface of the berries could be found the bluish freckles. Used fresh and dry (kishmish).

Qyrmyzy Saabi is one of the ancient table grape varieties of Nakhichevan and Western Azerbaijan. By its morphological and biological specifications *Ag meleyi* is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.) The bunches and berries are large, with the very beautiful appearance. Berries' pulp is consistent and crispy. Flavor is harmonic and nice. The peel is thick and elastic. The surface is pruinose. The color is red or dark red. When eating the peel is not felt. The plants are strong, good growing. The variety is also widely cultivated on the Apsheron peninsula. Thank to the beautiful appearance of the plants and berries the variety is also cultivated with the ornamental purposes. Ripening period is the late September. Consumed fresh. Suitable for transportation and storage.

Novrast is one of the most ancient local grape varieties of Azerbaijan. The old plants of this variety can be found in South-Western Azerbaijan and Nakhichevan. The variety was brought from the Western Azerbaijan by viticulturist scientist M.V.Amanov and placed in the collection of Azerbaijani Scientific Research Institute of Viticulture and Wine-making. By its morphological and biological specifications *Novrast* is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.). The early-ripe variety. The bunches and berries are large, with the beautiful appearance. The berries are inversely egg-shaped. The peel is thin, but durable and elastic not felt when eating. The pulp is fleshy, consistent and crispy. The flavor is harmonic and nice, with the specific taste. The berries contain little seeds. In some berries the seeds doesn't evolve and remain in the rudiment stage. Period of full ripening – late July or early

August. As a early-ripening variety is popular among viticulturists. Used fresh. The negative feature is the weak creation of adventive roots by one-year shoots. The plants and shoots are very strong.

Shahany is one of the ancient table grape varieties of Apsheron region. By its morphological and biological specifications *Shahany* is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.). The bunch is oblong, the berries large. The bunches and berries have the beautiful appearance. Fully ripened berries are dark-yellow or gold-yellow. The peel is thin and elastic. The pulp is consistent and weakly crispy. The seeds could be seen inside the fully ripened berries. The sugar content is very high. The flavor is harmonic and nice. Ripening period – early September. Suitable for storage. Used fresh. According the old literature sources, the variety deserved its name for very high quality (*Shahany* – qualified for Shah (king)).

Ag Khalily is one of the most valuable table grape varieties of Azerbaijan. By its morphological and biological specifications *Ag Khalily* is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.). One of the very early ripening varieties (first and second ten-day periods of July). Included in the standard assortment of Azerbaijan. *Ag Khalily* used to be one of the widely cultivated varieties of Azerbaijan. Synonyms – Ilinskiy, Yay uzum, *Ag novrast*, *Shah uzum*. The bunches and berries are in the medium size, with the nice appearance. The peel is thin, elastic, durable, not felt when eating. The pulp is relatively consistent, weakly crispy, weakly juicy, despite the average sugar content the flavor is harmonic and nice. Used fresh. Relatively less suitable for long-term storage and transportation.

Ag Darbandy is one of the ancient table grape varieties of Azerbaijan. *Ag Darbandy* variety is cultivated on the old vineyards in the ancient settlements of Apsheron region. The best plants are found in Binaqadi, Mehdiabad, Buzovna, Shagan, Shuvelan, Novkhany, Fatmai, Turkan, Mashtaqa, Zabrat and other settlements. By its morphological and biological specifications *Ag Darbandy* is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.). Very late ripening. The bunch and the berries are very large, with the nice appearance. The peel is thick, durable and elastic, not felt when eating. The pulp is fleshy, very consistent and crispy. The flavor is harmonic and nice. Suitable for long-term storage and transportation. The local

population stores the grapes on the plants and in the cellars for some months, sometimes up to March. The full ripening period is the late October. After the long period of storage on the plants the berries obtain the dark yellow or pink tint and look like the amber. Used fresh the bunches can weight 1 kg and more.

Mahmudu is one of the most ancient varieties of Salyan region. The variety is cultivated in most viticulture farms of Azerbaijan. By its morphological and biological specifications *Mahmudu* is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.). Thanks to its high productivity, relatively early ripening period, tolerance to diseases and pests, large bunches and berries, nice appearance, high flavor and quality characteristics the variety is popular among farmers. The bunches are oblong, the berries remaining for the long term on the plants retain their commercial, organoleptic and taste characteristics for long period. The peel is thick, durable and elastic, not felt when eating. The surface is covered with the thick wax layer. The pulp is consistent, weakly crispy, with the harmonic and nice flavor. Suitable for transportation and storage. Used mainly fresh.

Khan uzumu is one of the most ancient local grape varieties of Garabagh region. Cultivated in the old settlements of Garabagh. By its morphological and biological specifications *Khan uzumu* is related to eastern ecological-geographical group of *V.vinifera* species (*convar orientalis* Negr.). The bunches and berries have the nice appearance. The peel is thick, durable and elastic, not felt when eating. The surface is covered with the thick wax layer. When the wax surface is wiped out the colour is shining black. The flavor is nice. The pulp is consistent, crispy. All the berries in the bunch are in the same size. Full ripening in the late August – early September. Suitable for transportation and storage. May be stored on the plants for long period. Very high productive variety. The bunches are very large, 1,5 kg and more in weight. Used fresh and for preparing the raisins.

One hundred and sixty *V. vinifera* cultivars coming from the Caucasus region and Central and Eastern Europe were studied by 10 SSR loci. The allelic profiles per each locus were used to calculate descriptive statistics and the results according to the accessions geographic origin are listed in Table 2.

A total of 147 unique genotypes were detected. In each groups, synonymous were identified, except for the cultivars included in the outgroup. No samples showing an identical profile but different geographic

origin were discovered. One hundred and sixty-six alleles and an average of 16.6 alleles per locus were detected. The number of different alleles ranged between 6.900 (Moldavian samples) and 11.200 (Azerbaijani samples), while the number of effective alleles varied between 4.493 (European varieties) and 5.948 (Azerbaijani cultivars).

The mean value of H_o revealed by the analysis was high (0.769), ranging from 0.698 (Moldova) to 0.814 (Europe). The H_e values were very similar to H_o values, showing a mean value of 0.769 and range from 0.733 (Moldova) to 0.814 (Azerbaijan).

Table 2. Genetic diversity of Azerbaijani, Armenian, European, Georgian and Moldavian cultivars revealed by analysis of 10 SSR loci.

Origin	Nr. of samples	Nr. of different genotypes	N_a^a	N_e^b	H_o^c	H_e^d
Azerbaijan	42	41	11.200	5.948	0.768	0.814
Central Europe	22	22	7.200	4.493	0.814	0.751
Georgia	44	40	8.700	4.504	0.741	0.750
Moldova	23	20	6.900	4.573	0.698	0.733
Armenia	29	24	8.100	5.263	0.809	0.797
Total	160	147	8.420	4.956	0.766	0.769

^a Number of different alleles; ^b Number of effective alleles; ^c Observed heterozygosity; ^d Expected heterozygosity.

Two different methods were performed to identify the correlation among cultivars and the structure of genetic groups. The PCoA analysis was computed on based on the genetic distance matrix

obtained by SSR allelic profiles and the two principal coordinates of PCoA were plotted in a 2-D scattered plot (Figure 1).

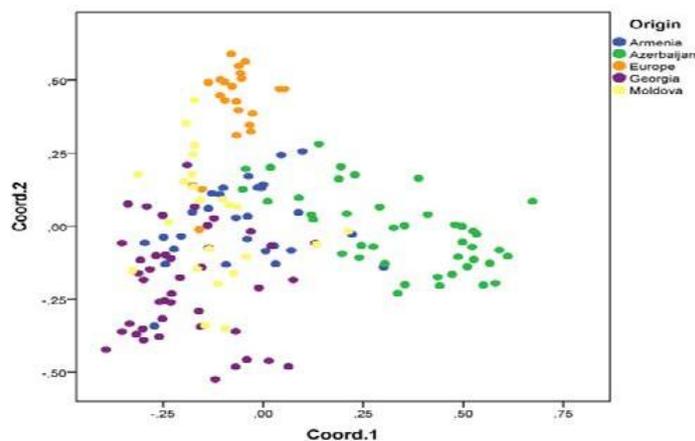


Figure 1. Plot of the first two principal coordinates of PCoA detected by SSR data for 160 samples coming from Azerbaijan, Armenia, Georgia, Moldova and Central Europe.

The first two principal coordinates (PC) accounted for 26.21 and 24.56% of the total variability. The distribution differentiated the samples into two main clusters: i) Azerbaijani groups, where the most part of samples coming from Azerbaijan were grouped; ii) the rest of Caucasian samples and the European (Central and Eastern) cultivars. Along the PC1, the samples coming from Azerbaijan were differentiated, while the outgroup cultivars appeared different along the PC2. Moreover, the presence of overlapping zones was recognized. Several samples from Azerbaijan appeared bordering some Eastern European (Moldova) and Caucasian accessions,

showing greater affinity. The group of samples more related to European and Caucasian varieties could be the gene pool originating the Western accessions, while the group of more different genotypes could be the gene pool associated to the enlarged center of primo-domestication, expanded to Central Asia regions (BACILIERI *et al.* 2013). The outgroup samples (Central European samples) were the samples showing the most significant differentiation in the Caucasian and East European groups. These overlapping zones disappeared removing the most two different groups (Azerbaijan and Central Europe) by

the dataset (data not shown) and the Armenian, Georgian and Moldavian groups appeared distinct.



Figure 2. Estimation of admixture proportions for 160 samples coming from Azerbaijan, Georgia, Moldova, Armenia and Central Europe by STRUCTURE analysis ($K = 3$).

The West-East gradient, following the grapevine migration from the first domestication centre, clearly identified in the analysis performed by S.Myles *et al.* [8] and S.Imazio *et al.* [10] was confirmed, despite clear overlapping areas slightly flattening the distribution. This differentiation of Azerbaijani samples could happen due to the different usage of grapes. Indeed, the Azerbaijani cultivars are table grape, because starting from 10th century AD, when the Azerbaijan became a Muslim country, the population started to select table grapes instead of wine grapes.

The second method used to infer the relationship among genotypes was the clustering algorithm implemented in STRUCTURE program. Different numbers of K populations were explored (Figure 2) and the optimal K value, evaluated following the EVANNO *et al.* (2005) method, estimated the most likely number of groups at $K = 3$. The threshold used to estimate the group assignation was $>80\%$ and about 91% of samples were assigned to a cluster at $K = 3$. The groups highlighted were: i) Central Europe group (92% of the outgroup samples); ii) an admixed group, containing the 73% of Armenian samples, 96% of Georgian genotypes and 89% of cultivars from Moldova; iii) Azerbaijani group (94% of cultivars). The samples showing the high admixture were the Armenian cultivars. Another STRUCTURE level was identified increasing the K value up to 5: one group per each geographical region was displayed (data not shown).

The genetic similarity among the Caucasian and European samples was also evaluated by Nei's genetic distance. The Nei's genetic distance values ranged from 0.242 and 0.845, respectively for the most similar groups - Armenia and Moldova and the most dissimilar groups - Central Europe and Georgia. The Moldavian samples, geographically closer to Central

Europe, showed an high genetic distance value with varieties coming from Central Europe. The dissimilarity of Azerbaijani cultivars was also confirmed by high values of Nei's genetic distance. Furthermore, the F_{st} values were consistent with results obtained by Nei's genetic distance. The values were very low, ranging from 0.034 (Armenia vs Moldova) and 0.087 (Central Europe vs Georgia).

4. Conclusions

The purpose of this study was to provide additional information about the genetic diversity of European and Caucasian varieties and the relationship among these different winegrowing areas, analyzing a large set of cultivars. According to our results, a clear connection between *proles pontica* and *proles orientalis* might be assumed, highlighting the high genetic diversity of Caucasian germplasm, considered a possible primary centre of grapevine domestication. Studied grape varieties are similar in many characteristics and differ in many others. Local grape varieties have a very wide range of diversity by their morphological characteristics. So, Azerbaijani grape varieties are distinguished by their color, shape and size of the berries, flavor and aroma, ripening period, direction of use, processing and storage specifications, etc.

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