# COMPARISON OF GENETIC DIVERSITY BETWEEN CULTIVATED AND WILD GRAPE VARIETIES ORIGINATING FROM THE NEAR-CASPIAN ZONE OF AZERBAIJAN

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## Abstract

**Aim**: The Caucasus is considered as a possible primary centre of origin of grapevine. The aim of our research work was to study genetic diversity among cultivated Caucasian grape varieties and wild relatives originating from Near-Caspian areas of Azerbaijan on the basis of simple sequence repeat (SSR) markers.

**Methods and Results**: Microsatellite allele data obtained in 31 local table grape varieties of *Vitis vinifera* L. ssp. *sativa* (Absheron region) and 34 wild varieties of *Vitis vinifera* L. ssp. *sylvestris* (Nabran, Davachi and Guba regions) were used to estimate population genetic parameters such as the polymorphism information content (PIC), the probability of identity (PI) and the frequency of null alleles (r). The genetic differences among the wild populations and between the wild and the cultivated gene pools were assessed using POPGENE, Arlequin and GENETIX programs. The selected fifteen microsatellite (SSR) markers revealed a high degree of polymorphism within and among the grape populations analyzed. The genetic similarity index ranged from 0.02 to 0.933.

**Conclusions**: Among the selected markers, VVMD28 and VVMD36 displayed the highest diversity level regarding the expected heterozygosity and PIC (highest values) and PI (lowest values). In consequence, we postulate that these two markers are the most appropriate ones for the identification of grape accession and the determination of genetic diversity among cultivated and wild grape genotypes. Clustering analysis based on SSR markers data led to a good separation between cultivated and wild accessions and between wild accessions originating from different regions.

**Significance and impact of the study**: The fifteen microsatellite markers used in this study were highly informative for the identification and analysis of genetic structure of Azerbaijan grapevine populations and clarified the relationships among grape accessions.

**Key words**: Azerbaijan wild grapes, AMOVA, genetic diversity, SSR markers, *Vitis vinifera* ssp. *sativa* and *Vitis vinifera* ssp. *sylvestris*

## Résumé

**Objectif**: Le Caucase est une zone géographique considérée comme un centre de diversité de *V. vinifera*. Le but de cette étude était d'étudier, au moyen de marqueurs microsatellites, la diversité génétique des variétés locales de vigne du Caucase, ainsi que celle des populations sauvages qui poussent en Azerbaïdjan, dans les régions proches de la mer Caspienne.

**Méthode et résultats**: En se basant sur ces données microsatellites obtenues sur 31 variétés locales de raisin de table et 34 individus sauvages appartenant à l'espèce *V. vinifera*, nous avons pu estimer différents paramètres tels que le PIC (polymorphic information content), la fréquence d'allèles nuls (r), le PI (probabilité d'identité). Pour estimer la diversité génétique à l'intérieur des compartiments sauvages et entre les compartiments sauvages et cultivés, nous avons utilisé les logiciels POPGENE, Arlequin et GENETIX. Nous avons pu montrer que les 15 marqueurs microsatellites avaient un niveau élevé de polymorphisme à l'intérieur et entre les populations analysées. L'index de similarité génétique variait entre 0,02 et 0,933.

**Conclusions**: Les marqueurs VVMD28 et VVMD36 ont révélé le plus fort taux d'hétérozygotie, de PIC et la valeur la plus basse de PI, et semblent donc être les plus adaptés pour l'identification des variétés et pour déterminer la diversité génétique à l'intérieur des populations cultivées ou sauvages de vigne. Une étude de structuration génétique basée sur les marqueurs microsatellites a mis en évidence une séparation des vignes cultivées et vignes sauvages, mais aussi une différenciation entre vignes sauvages de régions différentes.

**Signification et impact de cette étude**: Les quinze marqueurs microsatellites utilisés pour cette étude se sont révélés très informatifs pour mettre en évidence une structuration génétique des populations de vignes en Azerbaïdjan et aussi pour clarifier les relations entre les génotypes cultivés.

**Mots-clés**: vignes sauvages d'Azerbaijan, AMOVA, diversité génétique, marqueurs SSR, *Vitis vinifera* ssp. *sativa, Vitis vinifera* ssp. *sylvestris*

**Abbreviations**: SSR = Simple Sequence Repeat, UPGMA = Unweighted Pair Group Method with Arithmetic averages, AMOVA = Analysis of Molecular Variance

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## INTRODUCTION

Grapevine (*Vitis vinifera* L.) is an ancient fruit species and its domestication goes back to the 11th century B.C. (Vignani *et al.*, 2002). Over the past two centuries, many authors including De Candolle (1855), Vavilov (1935), Negrul (1965), Zhukovsky (1975), Olmo (1976), and Zohary and Hopf (1993) pointed out the relevance of the region between the Black Sea, the Caucasus and the Caspian Sea as a possible primary centre of origin of grapevine. The existence of a large number of native varieties well adapted to local conditions (*V. vinifera* L. ssp. *sativa*) and wild plant material (*V. vinifera* L. ssp. *sylvestris* Gmel.), which is represented by typical fluffy-leafed (typica Negr.) and naked-leafed (aberrans Negr.) forms, points out Azerbaijan as one of the centre of origin of grapevine (*V. vinifera*) (Negrul, 1973). Historical information and recent archaeological excavation confirmed this postulate (Negrul, 1973). The wild species *Vitis vinifera* ssp. *sylvestris*, the putative ancestor of the cultivated grapevine, is widely spread on the territory of Azerbaijan Republic from 18 metres below sea level (Kur riverside in Salyan area) to 2,000 metres above sea level (Gusar area). It is particularly present in the southern regions of the Caucasus Mountains, in the Talish Mountains, in the Tugay forest, on the valleys of Kur and Alazan rivers but also in the Azerbaijan autonomous province of Nakhchivan (Allahverdiyev, 1973). The diversity of natural conditions characterized by the mountainous and flat orography of the country (Negrul, 1946), differences in climates (existence of 9 to 11 climatic zones in Azerbaijan) (Budagov, 2007) and various soil conditions lead to the identification of abiotic and biotic stress resistant, morphologically different wild grape forms (Negrul, 1946), as well as to the cultivation of high-quality table and wine varieties. Those cultivars are also adapted to the local market, long-term conservation, long-distance transportation and drying conditions (Negrul, 1946).

The longtime history of grapevine cultivation has determined a complex picture in which many biotypes or even cultivars are misidentified or have different names in different world regions. This often makes genetic identification difficult. Grapevines are propagated by cuttings and the resulting clones are genetically identical to each other (except for somatic mutations) and to the mother plant (the original seedling from which cultivars and cuttings were derived) (Vignani *et al.*, 2002).

Ampelography is a traditional botanical method of grape classification based only on morphological traits and which cannot distinguish varieties displaying similar characters under the same ecological conditions. The evaluation of genetic diversity was enriched by the development of molecular marker technologies. The combination of both - molecular marker analysis and phenotypic evaluation, is a powerful tool for grouping genetically close genotypes and for sorting out genetically distinct parents for breeding programs. In the context of *ex situ* conservation, it has been demonstrated that molecular markers are very useful for the management of germplasm collections (Akkaya *et al.*, 1995 ; Decroocq *et al.*, 2003 ; Rossetto *et al.*, 2002 ; Westman and Kresovich, 1997). Among them, microsatellite markers became the most preferential and reliable tool for grapevine accession identification due to their high level of polymorphism, abundance in the genome, co-dominant nature and Mendelian inheritance. In many studies, SSR (microsatellite) markers have been used for the assessment of genetic diversity among wild and cultivated grape accessions and have been extensively used for the characterization of grapevine collections (Dangl *et al.*, 2001 ; Ibanez *et al.*, 2003 ; Lopes *et al.*, 1999 ; Martin *et al.*, 2003 ; Ortiz *et al.*, 2004).

The aim of our research work was to study genetic diversity among local Caucasian grape varieties and their wild relatives originating from Near-Caspian areas of Azerbaijan on the basis of SSR markers.

## MATERIALS AND METHODS

### 1. Plant material

A total of 65 accessions, comprising 31 local table grape varieties and 34 wild varieties of *Vitis vinifera* originating from the Near-Caspian zone of Azerbaijan, were analyzed (figure 1). The plant materials were sampled from the grape collection of the Genetic Resources Institute of the Azerbaijan National Academy of Sciences and from the Azerbaijan Sciences Research




**Figure 1 - Map showing the 4 studied grapevine growing regions.**


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Institute of Viticulture and Wine-making. Local and wild accessions used in this study are listed in table 1.

## 2. DNA extraction

Total plant DNA was isolated from cambium tissues using a modified version of the CTAB protocol described by Doyle and Doyle (1990). Briefly, 1 gr of green tissues scrapped from dormant woods was powdered using a mortar and pestle under liquid nitrogen and suspended in 5 mL of extraction buffer 1 [0,2 M Tris-HCl (pH 8.0), 0.07 M EDTA (pH 8.0), 2 M NaCl, 0.02 M $Na_2S_2O_5$]. An aliquot of 500 µL was transferred into an Eppendorf tube and mixed with 450 µL of buffer 2 [2 % cetyl trimethyl ammonium bromide (CTAB), 1.4 M NaCl, 0.02 M EDTA (pH 8.0), 0.1 Tris-HCl (pH 8.0)]. After thorough agitation using a vortex, the mix was incubated for 1 hour at 65 °C in a water bath (with occasional mixing) and then centrifuged at 10,000 g for 5 min at 4 °C. The supernatant was transferred into a new tube and mixed with 450 µL chloroform/octanol (24:1). After centrifugation at 10,000 g for 15 min at 4 °C, 300 µL of supernatant was mixed with 300 µL isopropanol (-20 °C) and 150 µL of 10 M $CH_3COONH_4$ and DNA was precipitated at -20 °C for 25 min. Following centrifugation at 10,000 g for 10 min at 4ºC, the precipitated DNA was washed in 750 µL of 70 % ethanol. The DNA pellet was dried in a Speed-Vac (Mandel Scientific Company) and dissolved in 200 µL 0.1X TE [1 mM Tris-HCl pH=8.0, 0.1 mM EDTA pH=8.0] at 4 °C overnight.

## 2. Microsatellite amplification

Fifteen pairs of primers corresponding to selected nuclear microsatellite loci, namely VVMD5, VVMD6, VVMD7, VVMD8, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD31, VVMD32, VVMD36 (Bowers *et al.*, 1996, 1999), VVS2 (Thomas *et al.*, 1998), VrZAG62 and VrZAG79 (Sefc *et al.*, 1999), were used. The markers were chosen based on their ability to reveal high levels of polymorphism in previously published

**Table 1 - Name and origin of grapevine accessions selected in the study.**

| Cultivated samples | | | Wild samples | |
|---|---|---|---|---|
| № | Name | Origin | № | Origin |
| 1 | Davachi Halimasi | Abşeron | 32 | Nabran |
| 2 | Fikrati | Abşeron | 33 | Nabran |
| 3 | Malahati | Abşeron | 34 | Nabran |
| 4 | Farashi | Abşeron | 35 | Nabran |
| 5 | Sharabi | Abşeron | 36 | Davachi |
| 6 | Tozlayiji | Abşeron | 37 | Davachi |
| 7 | Nardaran Darbandisi | Abşeron | 38 | Davachi |
| 8 | Ag uzum | Abşeron | 39 | Davachi |
| 9 | Sarigila tezyetishan | Abşeron | 40 | Davachi |
| 10 | Ag gulabi | Abşeron | 41 | Davachi |
| 11 | Danaguzu | Abşeron | 42 | Davachi |
| 12 | Gizil uzum | Abşeron | 43 | Guba |
| 13 | Haji Abbas | Abşeron | 44 | Davachi |
| 14 | Sikhgara | Abşeron | 45 | Davachi |
| 15 | Absheron kishmishi | Abşeron | 46 | Guba |
| 16 | Uzunsov gilali ag shani | Abşeron | 47 | Guba |
| 17 | Gavangir | Abşeron | 48 | Davachi |
| 18 | Iri gilali ag shani | Abşeron | 49 | Guba |
| 19 | Ag shani | Abşeron | 50 | Davachi |
| 20 | Absheron galinbarmagi | Abşeron | 51 | Guba |
| 21 | Fatmayi | Abşeron | 52 | Davachi |
| 22 | Sarigila ortayetishan | Abşeron | 53 | Davachi |
| 23 | Sarigila gejyetishan | Abşeron | 54 | Davachi |
| 24 | Absheron kechimamasi | Abşeron | 55 | Davachi |
| 25 | Shireyi | Abşeron | 56 | Davachi |
| 26 | Gara shani | Abşeron | 57 | Guba |
| 27 | Shabrani | Abşeron | 58 | Davachi |
| 28 | Ala shani | Abşeron | 59 | Guba |
| 29 | Ag shani (clone) | Abşeron | 60 | Guba |
| 30 | Absheron khatinisi | Abşeron | 61 | Guba |
| 31 | Iri salkhim | Abşeron | 62 | Nabran |
| | | | 63 | Nabran |
| | | | 64 | Nabran |
| | | | 65 | Nabran |


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studies. PCR reactions were carried out with a Gene Amp 9700 Applied Biosystems thermocycler. Each PCR reaction was carried out using 6 ng of template DNA in 20 µL total reaction volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01 % gelatine, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 mM of each primer and 0.5 unit *Taq* DNA polymerase. Reaction cycles were as follows: 5 min at 94 °C followed by 40 cycles of 30 s at 94 °C, 30 s at individual hybridization temperature (see below), and 30 s at 72 °C. The program ended with a 30 min incubation at 72 °C. The individual hybridization temperatures were as follows: VVMD5 56 °C, VVMD6 52 °C, VVMD7 52 °C, VVMD8 54°C, VVMD21 56 °C, VVMD24 56 °C, VVMD25 56 °C, VVMD27 56 °C, VVMD28 56 °C, VVMD31 56 °C, VVMD32 56 °C, VVMD36 56 °C, VVS2 50 °C, VrZAG62 50 °C and VrZAG79 50 °C.

After amplification, PCR products were checked on 1.5 % agarose gels prior to running on polyacrylamide denaturing gels. PCR samples were denatured in 50 % formamide, 10 mM EDTA (pH 8.0), 0.05 % Bromophenol blue, and 0.05 % Xylene cyanol by incubation for 5 min at 94°C. Two µL of each sample was run on denaturing polyacrylamide sequencing gels [6 % w/v acrylamide-bisacrylamide (19:1), 10 M urea, 0.5X TBE (T4415, Sigma)] at 80 W for 3 to 3.5 hours depending on the length of the DNA fragments. After running, the gels were fixed in a 10 % acetic acid solution for 20 min, washed 3 times in distilled water, silver stained in 0.1 % $AgNO_3$ and 0.06 % formaldehyde for 1 hour, and revealed in sodium carbonate solution (3 % $Na_2CO_3$, 0.06 % formaldehyde, 0.13 % $Na_2S_2O_3$). The product size was determined with 10 and 50 bp DNA ladder standards (Invitrogen). Each PCR sample was analyzed at least twice to confirm genotype reproducibility.

## 3. Statistical analysis

Diversity in *V. vinifera* with SSR markers. The following parameters were estimated based on microsatellite allele data: number of alleles per locus ($N_a$), effective number of alleles ($N_e$), observed heterozygosity ($H_o$), expected heterozygosity ($H_e$), and deviation from Hardy-Weinberg equilibrium. The polymorphic information content (PIC) (Botstein *et al.*, 1980), the probability of identity (PI) (Paetkau *et al.*, 1995) and the frequency of null alleles (r) (Brookfield, 1996) were estimated based on the following equations:

$$PIC = 1 - \sum p_i^2 - 2\left[\sum_{i=1}^{n-1}\sum_{j=i+1} p_i^2 p_j^2\right]$$

$$PI = \Sigma P_i^4 + \Sigma\Sigma (2P_iP_j)^2$$

$$r = (H_e - H_o) / (1 + H_e)$$

where $P_i$ and $P_j$ are the frequencies of the $i^{th}$ and $j^{th}$ alleles within each locus, respectively. A dendrogram representing genetic similarity among samples was constructed using UPGMA from the genetic distance matrix. These analyses were performed using the SPSS software package version 2003 12.0 (IBM, Chicago).

Comparison of the cultivated and wild gene pools. The above mentioned genetic diversity parameters were calculated on the two gene pools. Additionally, several parameters were estimated to assess the genetic differences among the wild populations and between the wild and the cultivated gene pools. The genetic distance (Nei, 1978) between cultivars and wild accessions was calculated using the POPGENE program version 1997 1.1 (University of Alberta, Edmonton, Canada). First, the genetic structure of cultivated and wild groups was

**Table 2 - Genetic markers (15 microsatellite loci) and parameters used for the analysis of the 65 Azerbaijan grapevine accessions.**

| Locus | $N_a$ | $N_e$ | $H_e$ | $H_o$ | PI | PIC | r |
|---|---|---|---|---|---|---|---|
| VVMD5 | 12 | 14.97 | 0.799 | 0.754 | 0.061 | 0.764 | 0.02 |
| VVMD6 | 9 | 3.17 | 0.68 | 0.631 | 0.13 | 0.645 | 0.03 |
| VVMD7 | 10 | 4.84 | 0.79 | 0.985 | 0.066 | 0.751 | -0.1 |
| VVMD8 | 11 | 4.87 | 0.79 | 0.492 | 0.065 | 0.73 | 0.17 |
| VVMD21 | 11 | 3.45 | 0.71 | 0.723 | 0.12 | 0.61 | -0.01 |
| VVMD24 | 6 | 3.11 | 0.68 | 0.723 | 0.143 | 0.63 | -0.07 |
| VVMD25 | 12 | 4.67 | 0.79 | 0.615 | 0.067 | 0.71 | 0.097 |
| VVMD27 | 14 | 4.56 | 0.786 | 0.677 | 0.047 | 0.72 | 0.06 |
| VVMD28 | 18 | 9.63 | 0.892 | 0.8 | 0.018 | 0.855 | 0.048 |
| VVMD31 | 9 | 4.34 | 0.769 | 0.754 | 0.088 | 0.63 | 0.025 |
| VVMD32 | 13 | 8.06 | 0.876 | 0.815 | 0.027 | 0.76 | 0.032 |
| VVMD36 | 20 | 9.13 | 0.89 | 0.738 | 0.019 | 0.81 | 0.08 |
| VVS2 | 15 | 9.23 | 0.89 | 0.877 | 0.045 | 0.8 | 0.007 |
| VrZAG62 | 9 | 6.85 | 0.85 | 0.769 | 0.039 | 0.75 | 0.04 |
| VrZAG79 | 15 | 6 | 0.83 | 0.892 | 0.038 | 0.734 | -0.03 |
| Mean | 12.26 | 5.79 | 0.802 | 0.749 | 0.065 | 0.726 | 0.052 |

$N_a$-number of alleles; Ne-number of efficiency alleles; He-expected heterozygosity; Ho-observed heterozygosity; PI-probability of identity; PIC-polymorphic information content; r-estimated frequency of null alleles.


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examined by analysis of molecular variance (AMOVA). For this purpose, AMOVA was carried out for determining genetic difference within populations as a first source of variance, among Guba, Nabran and Davachi wild accessions as a second source of variance, and among cultivated samples and the wild genotypes as a third source of variance (Excofier *et al.*, 1992) using the Arlequin software package version 2000 2.0 (University of Geneva, Geneva). Second, the $F_{ST}$ coefficient was employed as a measure of genetic differentiation among populations and estimated using the GENETIX program version 1999 4.04 (University of Montpellier II, Montpellier, France). It was calculated among the wild populations and between the wild and cultivated gene pools.

## RESULTS AND DISCUSSION

### 1. Marker efficiency

The selected fifteen microsatellite markers revealed a high degree of polymorphism among the grape populations analyzed. A total of 184 different alleles were found among the 65 samples. Parameters for the evaluation of genetic diversity included the number of alleles, the effective number of alleles, observed heterozygosity, expected heterozygosity, probability of identity, estimated frequency of null alleles as well as polymorphic information content (PIC) (table 2). These estimators of diversity differed for each microsatellite marker. Indeed, the number of alleles per locus (Na) ranged from 6 for VVMD24 to 20 for VVMD36, with an average of 12.26 alleles per locus for the 15 loci. The effective number of alleles per locus (Ne) ranged from 3.11 for VVMD24 to 14.97 for VVMD5, with an average of 5.79 for the 15 loci. Those data are in concordance with previous studies performed in grape (Lopes *et al.*, 1999 ; Scott *et al.*, 2000 ; Sefc *et al.*, 2000 ; 2001 ; Thomas *et al.*, 1998). The difference between the average number of alleles and effective number of alleles per locus indicates that rare alleles are detected in a rather limited number of individuals. Moreover, while comparing the expected heterozygosity (He) and PIC parameters, the VVMD24 locus showed the lowest diversity whereas VVMD28, VVMD36 and VVS2 showed the highest diversity. According to Najafi *et al.* (2006) and Riccardi *et al.* (2006) who studied genetic diversity among Iranian and European grape accessions, the VVMD28 and VVMD5 and VVMD28 and VVMD34 loci showed the highest diversity, respectively.

The major application of microsatellite markers in viticulture is the identification and differentiation of varieties. Therefore, if a marker is accurate in the identification and differentiation of various samples, it is expected to be suitable for genetic studies. Considering the diffusion of alleles in the investigated genotypes, the PI values were low (0.018-0.143) (table 2). These results are in concordance with data presented by Lopes *et al.* (1999) and Sefc *et al.* (2000). Since the VVMD28 and VVMD36 loci not only showed the highest diversity among Azerbaijan grape samples but also showed the lowest PI values, they are therefore recommended to distinguish varieties and determine genetic diversity between wild and cultivated grape material.

Moreover, the ultimate goal in breeding programs is the production of heterozygous individuals in order to exploit heterosis. The observed heterozygosity at the VVMD8 locus was significantly below the expected level which is probably due to the occurrence of null alleles at this locus. This hypothesis was confirmed by calculating the probability of null alleles (r) for this locus, using the equation proposed by Brookfield (1996). The "r" value was significantly higher for VVMD8 compared to the other loci (table 2). However, we cannot rule out other factors like limitations of interference and convergence between similar individuals, or the presence of low gene flow at this locus. These primers can also be recommended to be used in study of species identification (Dzhambazova *et al.*, 2007 ; Klein *et al.*, 2008 ; Pollefeys and Bousquet, 2003, This *et al.*, 2004).

Calculation of deviation from Hardy-Weinberg equilibrium showed that all of the loci except VVMD7 deviated from Hardy-Weinberg equation in each population under examination (data not shown). Among them, VVMD8 and VVMD21 showed deviation from Hardy-Weinberg equation in all populations tested. Furthermore, cultivated grape individuals originating from Absheron and wild grape material sampled in Guba displayed the highest and the lowest deviation ratios from Hardy-Weinberg equation, respectively. Three main hypotheses exist in the literature to account for heterozygote deficiency within populations (Morand *et al.*, 2002) : mating with relatives, null alleles at microsatellites and Whalund effect (existence of subunits within each population). In the present case, we can not exclude the co-occurrence of these different effects.

### 2. Determining genotypic similarities

Cluster analysis for all samples was performed according to Jaccard's similarity index following the UPGMA method. It allowed to classify all the genotypes into nine main clusters (figure 2). The first cluster consisted of 30 of the 31 cultivated genotypes. Indeed, the cv. Nardaran Darbandisi grouped in another cluster, at a rather short distance from other cultivated genotypes, which shows the genetic distance between this genotype and other cultivars. Similarly, all wild genotypes from Guba grouped in the 8$^{th}$ cluster except for one sample (N°46) which grouped separately in the 7$^{th}$ cluster, demonstrating its genetic divergence from the other Guba


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samples. Although the fourth, sixth and eighth clusters correspond to one or two members of wild Davachi genotypes, as well as the third and ninth clusters each consisting of only one wild Davachi genotype, most individuals originating from this region, in total 10 samples, grouped in the 7th cluster. In comparison, the 4th cluster contained 9 genotypes which made up 13.85 % of all examined genotypes, all originating from the Nabran region with the exception of one genotype (N°58) from the Davachi region. In regard to the above data, we can conclude that the observed genetic diversity in grape cultivated and wild material sampled in Azerbaijan is geographically structured.

The two samples "Gara shani" and "Ala shani" showed identical genotypes at all loci tested and were then undistinguishable in our study (data not shown). Similar SSR profiles were also obtained for "Sarigila ortayetishan" and "Sarigila gejyetishan" and therefore the markers used for grapevine identification fail to discriminate these two samples. Because there is no available genealogy for these varieties, no exact statement can be made on these samples. They are described under

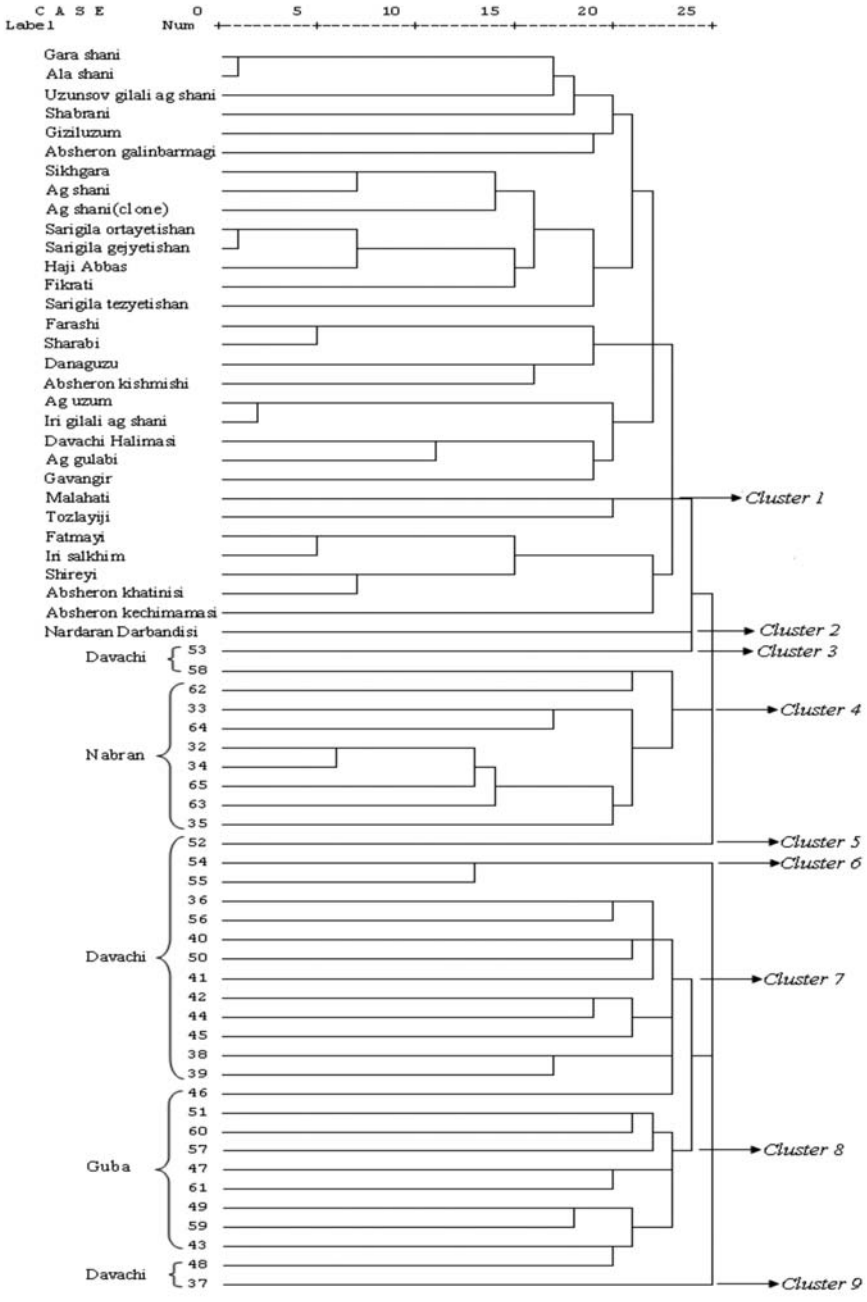



**Figure 2 - Dendrogram showing the genetic relationship among wild and cultivated Azerbaijan grapevines.**
The scale is based on Jaccard's similarity index.


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different names (synonym varieties); however, there is a probability that each of these pairs have a common ancestor or are clones of the same variety. This is highly probable especially for the Sarigila ortayetishan and Sarigila gejyetishan cultivars, their only difference being their ripening time (middle and late ripening, respectively). Even though there is for the moment no comparable study in the literature using that many markers to analyse and determine grape diversity, we believe that increasing the number of SSR markers in order to cover the grape genome will improve the accuracy of this study. It should reveal more differences between grape samples, especially between genetically close genotypes.

Among all investigated genotypes, the highest genetic similarity was observed between the cultivars Ag uzum and Iri gilali ag shani with a similarity index of 0.933. The Fatmayi and Iri salkhim cultivars as well as the Farashi and Sharabi cultivars appeared also very close genetically with a similarity index of 0.852 and 0.833, respectively. The grape individuals showing the lowest level of similarity within the cultivated samples were the Ag gulabi and Absheron khatinisi cultivars, with a similarity index of 0.058. Within the wild grape genotypes, the highest genetic similarity was observed between samples N°32 and N°34 (similarity index of 0.8), while the lowest genetic similarity was detected between samples N°53 and N°56 (similarity index of 0.02). The average genetic similarity among the cultivated samples was higher than the average similarity index among wild genotypes. This data demonstrates that wild grape samples are more distant between them than cultivated ones.

### 3. Genetic diversity within populations

Parameters of genetic diversity for various Azerbaijan grape populations are indicated in table 3. The highest genetic diversity depicted by the expected heterozygosity value was observed in the wild grape population from the Davachi region. Furthermore, this population of 17 members displayed the highest average number of alleles and effective number of alleles. Although the number of cultivars sampled in the Absheron peninsula was approximately twice the number of wild varieties sampled in the Davachi region, the diversity of this wild population was higher than of the cultivated population. One explanation is the selection pressure of domestication causing a decrease of diversity at some loci among cultivated material. Alternatively, it could be a founder effect in which populations of cultivated samples are initially originating from a limited number of genitors selected among ancient populations. However, since the difference in numbers of individuals sampled in each region (from 8 in the Nabran region to 17 in the Davachi region) might impair the calculation of genetic diversity within wild populations, the present study should be completed in the future by the analysis of similar sized wild populations from the different Azerbaijan regions, giving a better idea of the genetic diversity and its structure all over the country.

### 4. Genetic differentiation among populations

Analysis of molecular variance (AMOVA) was performed at three levels. First, the populations of wild and cultivated samples were examined and the results of AMOVA indicated a significant difference between wild and cultivated samples. Second, wild samples from three different regions were examined, revealing a significant difference between samples from different regions. Finally, the difference between samples of each of the 4 populations was examined and again, AMOVA data indicated significant differences between samples.

The AMOVA results also showed that despite the significant difference between the samples in the 4 populations, the highest genetic difference was observed between wild and cultivated populations (43.2 %) and then among the wild populations, between samples from Nabran, Guba and Davachi (38.2 %) (table 4).

The estimation of the genetic differentiation among populations ($F_{ST}$) was calculated as described in Materials and Methods. It confirmed the difference detected above among wild populations and between wild and cultivated genetic pools. As depicted in table 5, the samples originating from Nabran were the most divergent from the samples from all the other Azerbaijan regions. In fact, the samples from Nabran and Guba showed the highest difference. Moreover, Guba and Davachi individuals displayed the highest genetic similarity. This similarity was also observed in cluster analysis (figure 3).

**Table 3 - Genetic parameters averaged over all 15 loci for each investigated grape population.**

| Population | $N_a$ | $N_e$ | $H_o$ | $H_e$ | N |
|---|---|---|---|---|---|
| Cultivated (Absheron) | 7.73 | 4.64 | 0.8 | 0.735 | 31 |
| Wild (Nabran) | 4.6 | 3.26 | 0.69 | 0.63 | 8 |
| Wild (Guba) | 5.67 | 3.98 | 0.67 | 0.71 | 9 |
| Wild (Davachi) | 8.33 | 4.9 | 0.73 | 0.78 | 17 |
| All wild populations | 6.2 | 4.05 | 0.7 | 0.71 | |

$N_a$-actual number of alleles; $N_e$-effective number of alleles; $H_o$-observed heterozygosity; $H_e$-expected heterozygosity; N-number of genotypes.


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Figure 3 shows the dendrogram resulting from the matrix of genetic distance for the studied populations. Samples originating from Nabran clearly grouped separately, apart from samples from all the other Azerbaijan regions, while the individuals of Davachi and Guba regions grouped together and were separated by the lowest distance (0.84).

Thus, some features like high diversity, repeatability, co-dominant heredity and their presence across the genome, have made SSR markers an invaluable tool for many genetic studies such as identification of genotypes and varieties, determination of diversity and description of the genetic structure of population, determination of phylogenic relationships and management of gene banks and germplasm collections.

## CONCLUSION

In this study, for the first time, the genetic diversity of cultivated and wild grapevine accessions originating from Azerbaijan has been investigated with SSR markers. Our data showed that SSR markers are a powerful tool for grape accession identification and analysis of genetic structure of grapevine populations. The fifteen microsatellite markers used in this study were highly informative for the analysis and distinctness of Azerbaijan grapevine genotypes. This report demonstrated a large genetic diversity among cultivated and wild Azerbaijan grape populations. On the basis of genetic similarity, cultivated and wild individuals clustered in separate groups and several distinct genetic pools were revealed. We also showed the significant importance of geographic origin in the structuration of the Azerbaijan grape diversity pools. The genetic characterization of Azerbaijan cultivars revealed that "Ala shani" and "Gara shani", as well as "Sarigila ortayetishan" and "Sarigila gejyetishan", are synonyms. Such data is very useful for further management of Azerbaijan grape germplasm. Statistic analysis confirmed a significant genetic difference among wild populations and between wild and cultivated genetic pools; this will be used in future breeding programs.

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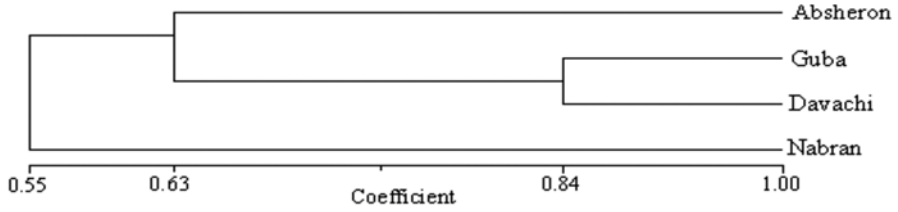



**Figure 3 - Dendrogram showing the genetic distance of the investigated Azerbaijan grape populations. The distance was calculated according to Nei (1978).**

**Table 4 - Analysis of molecular variance (AMOVA) based on the fifteen SSR markers.**

| Source of variation | Degree of freedom | Variance component | Percent of variance (%) | P-value |
|---|---|---|---|---|
| Within population | 61 | 2.18 | 18.6 | < 0.00 |
| Among wild populations | 2 | 4.49 | 38.2 | < 0.00 |
| Among wild and cultivated populations | 1 | 5.08 | 43.2 | < 0.00 |
| Total | 64 | 11.75 | | |

**Table 5 - Genetic differentiation between grapevine samples using pairwise values of $F_{ST}$ (up) and Nei genetic distance (low).**

| Samples | Absheron | Nabran | Guba | Davachi | All wild samples |
|---|---|---|---|---|---|
| Absheron | ----- | ----- | ----- | ----- | 0.078* |
| Nabran | 0.59 | ----- | 0.14* | 0.053* | |
| Guba | 0.63 | 0.55 | ----- | 0.043* | |
| Davachi | 0.64 | 0.59 | 0.84 | ----- | |

* significant at 0.05 (P-value< 0.05)


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