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COMPARISON OF AMPELOGRAPHIC AND MOLECULAR DIVERSITY OF AZERBAIJAN CULTIVATED AND WILD GRAPE VARIETIES

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To estimate genetic relationships among cultivated (*Vitis vinifera* ssp. *sativa*) and wild grapevine accessions (*Vitis vinifera* ssp. *sylvestris*) originating from regions near the Caspian Sea of Azerbaijan, comparison of ampelographic and ISSR markers diversity was performed. The genetic diversity of studied grapevine accessions at the phenotype and genotype levels is not geographically structured. Within the analyzed grapevine populations the highest genetic diversity is observed for the cultivated samples from the Absheron peninsula. Although the wild population separated from cultivated by ampelographic traits, the genetic similarity at the molecular level between wild and cultivated gene pools is observed. These findings suggest that the Azerbaijan cultivated grapevine population comes directly from wild population of these areas and this geographic region seems to be one of the possible primary centers of origin of *V.vinifera*.

Keywords: *Vitis* spp., ampelography, genetic distance, genetic similarity, ISSR markers

Introduction

The *Vitis vinifera* L. is one of the oldest cultivated plants and is thought to have originated in the region between the Mediterranean basin and the Caspian Sea [11]. Azerbaijan is one of the richest regions of the Globe for the wealth of grapevine grades. The occurrence of wild and cultivated vine in Azerbaijan dates back to very remote times of ancient history. Paleontological evidence and historical information of different periods indicate that there has been 7 thousand years of vine cultivation in Azerbaijan [10].

Traditional methods for description of grapevine varieties based on ampelography have contributed greatly to establishing the identity and relationships among *V. vinifera* cultivars. Nevertheless, ampelographic traits are often plastic, with a great [genotype × environment] components interaction rendering them less useful in classifying closely related cultivars. Last decade, molecular markers have proved to be a valuable tool for genetic studies and cultivar characterization. ISSR (Inter Simple Sequence Repeats) has been widely used for varietal fingerprinting or genetic diversity analysis, characterization of genetic relatedness among popula-

tions, detection of clonal variation, cultivar identification, detection of genomic instability and assessment of hybridization [2].

This study was aimed to estimate the genetic diversity between wild and cultivated grape varieties from the Near-Caspian zone of Azerbaijan, using ampelography and ISSR markers.

Materials and methods

A total of 60 local grapevine accessions, comprising 30 cultivars from Absheron peninsula (№1-30; Davachi Halimasi, Fikrati, Malahati, Farashi, Sharabi, Nardaran Darbandisi, Ag uzum, Sarigila tezyetishan, Ag gulabi, Dana gozu, Absheron gizil uzumu, Haji Abbas, Sikhgara, Absheron kishmishi, Uzunsov gilali ag shani, Gavangir, Iri gilali ag shani, Ag shani, Absheron galinbarmagi, Fatmayi, Sarigila ortayetishan, Sarigila gejyetishan, Absheron kechimamasi, Shireyi, Gara shani, Shabrani, Ala shani, Ag shani [clone], Absheron khatinisi and Iri salkhim, respectively) and 30 wild types (№31-34 from Nabran, №35-41, 43, 44, 47, 49, 51-55 and 57 from Shabran, №42, 45, 46, 48, 50, 56 and 58-60 from Guba) were analyzed. All plant materials were taken from the grape collection of National Gene Bank of Azerbaijan

Republic and the Azerbaijan Scientific Research Institute of Viticulture and Winemaking.

For each accession 23 morphological traits (length of annual bud, length and width of leaf, length of stalk of leaf, length of central vessel, number of bunches per bush, length and width of bunch, length of stalk of bunch, number of berries per bunch, length and width of berry, weight of bunch, number of seeds, length and width of seed, weight of 100 berries, weight of 100 seeds, number of fertile buds, number of green buds (this trait has not measured in wild grape), yield of bud, yield of per bush and time of vegetation) were measured according to the international descriptors for grape [5]. Data were analyzed using MSTATC [9] and SPSS v.12.0 software packages.

Total plant DNA was isolated from green tissues of cuttings using a modified version of the CTAB protocol [14] described by Doyle and Doyle [4]. Each PCR reaction was carried out using 50 ng of template DNA in 25 µL total reaction volume containing buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂], 5 mM MgCl₂, 10 mM of each dNTP, 10 mM primer and 0.5 unit *Taq* DNA polymerase. Reaction cycles were as follows: 5 min at 94°C followed by 35 cycles of 1 min at 94°C, 2 min at 50°C and 5 min at 72°C. The program ended with 10 min incubation at 72°C. Amplification products were separated by electrophoresis using 2% agarose gels with 1x TBE buffer at 100 V during 2 h. After running, the gels were stained with ethidium bromide (0.05%) and visualised with a Gel Doc camera. A cluster analysis was performed using the PAST software package [6]. Polymorphic information content (PIC) values were calculated for each ISSR primers according to the formula: $PIC = 1 - \sum (P_{ij})^2$, where P_{ij} is the frequency of the i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers [1].

Results and discussion

Ampelographic clustering of cultivars.

To determine the ampelographic and molecular

relationships of Azerbaijan wild and cultivated grapevines originating from the Near-Caspian zone, cluster analyses according to Euclidean distance and Jaccard's similarity index, respectively, were performed. The analysis based on morphological traits revealed three main groups. Although the cultivated and wild individuals are clustered in separate groups (excepting Nardaran Darbandisi), the observed genetic diversity is not geographically structured: 29 wild samples (out of 30) are grouped in the first cluster; the second cluster contains four cultivated grapevines (Haji Abbas, Gavangir, Sarigila gejetishan and Ala shani), as well as the wild sample 34; the third cluster includes remaining 25 cultivated grapevine accessions.

ISSR polymorphism. The nuclear genomes of 60 grape accessions were studied using 5 ISSR primers. Totally, we amplified 51 DNA fragments, where 45 samples were polymorphic (table 1). Overall, the number of amplicons ranged from 9 (for UBC 857 and UBC 873) to 12 (for UBC 827). However, the average number of bands and average number of polymorphic bands were 10.2 and 9, respectively, and the polymorphism level ranges from 66.66% (for UBC 873) to 100% (for UBC 827 and UBC 857), with an average polymorphism of 87.69% across all varieties.

Four primers used (out of five; excepting UBC 873) contained dinucleotide repeats, where nucleotides at the 5'- and 3'- ends of the primer play an important role in detecting polymorphism [3]. Four primers had anchors at the 3' end. The primer UBC 873 missing the terminal single base anchor was least polymorphic (66.66%), while those with two bases anchor at the 3' end were most polymorphic. ISSR markers showed relatively high level of polymorphism in the examined gene pools: the PIC values varied in the range of 0.862 (UBC 873) to 0.941 (UBC 811), where an average PIC value across all genotypes was 0.91 (for more details see table 1).

Table 1

ISSR primers used in the study and the values of some genetic parameters

ISSR primers	Primers' sequence (5'-3')	NAB	NPB	%P	PIC
UBC 810	(GA) ₈ T	11	9	81.81	0.93
UBC 811	(GA) ₈ C	10	9	90	0.941
UBC 827	(AC) ₈ G	12	12	100	0.89
UBC 857	(AC) ₈ YG	9	9	100	0.925
UBC 873	(GACA) ₄	9	6	66.66	0.862
Total	-	51	45	-	-
Min	-	9	6	66.66	0.862
Max	-	12	12	100	0.941
Average	-	10.2	9	87.69	0.91

NAB-number of amplified bands; NPB-number of polymorphic bands; %P- percentage of polymorphism; PIC-polymorphic information content

ISSR clustering of cultivars. A dendrogram representing genetic similarity among all selected samples is demonstrated in figure 1. For the Jaccard's genetic similarity index, the highest level of genetic similarity (GS=0.9) was revealed between the middle- and late-ripening Sarygile's (Sarigila ortayetishan and Sarigila gejyetishan) in seven

cluster, and also between wild samples 49 and 6 (cluster 11) from Shabran and Guba regions, respectively with a similarity index 0.86. By contrast, among all samples, the highest level of genetic distance (GS=0.13) was detected between cultivated grapes Ag shani (clone) and wild genotype №43 sampled from Shabran region.

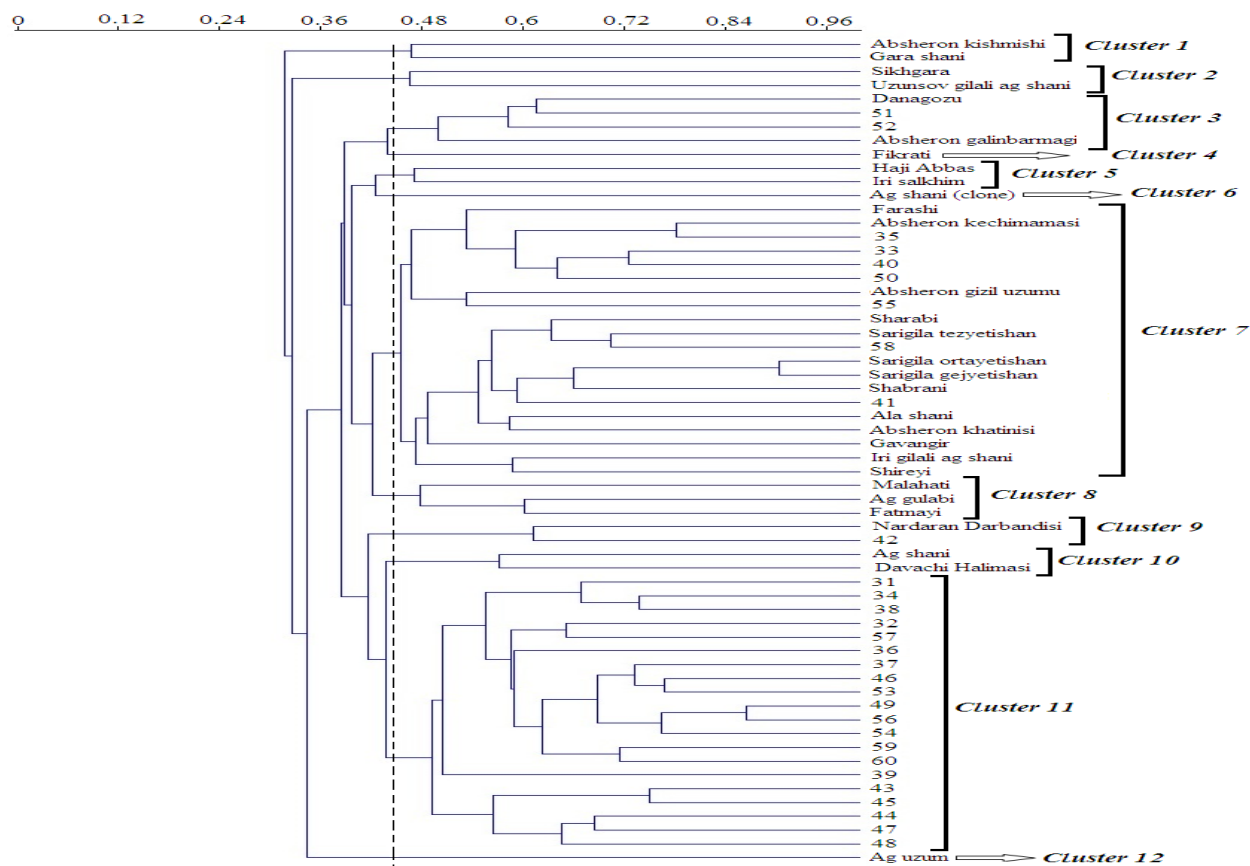


Fig. 1. Dendrogram showing the genetic relationships among cultivated and wild Azerbaijan grapevines based on ISSR markers

In dendrogram, all the genotypes are divided into twelve separate groups at a similarity index 0.45. The first, second, fifth, ninth and tenth clusters were assigned two numbers of cultivated genotypes for each (except the ninth cluster, which consisted of cultivated Nardaran Darbandisi and wild sample №42, originating from Guba region with the genetic similarity index 0.61 between them) and the forth, sixth and twelfth clusters included only one cultivated accession originating from Absheron peninsula; the eighth cluster also includes a minimum number of cultivars (Malahati, Ag gulabi and Fatmayi) belonging to the separate groups. This observation may be explained by the genetic distance of these samples from the other investigated cultivated and wild genotypes. On the contrary, the eleventh group contains bands for only 20 wild genotypes (3 Nabran, 6 Guba and 11 Shabran samples). Other wild genotypes belong to the third or seventh clusters. Some studies suggest that the intraspecies genetic diversification of *V. vinifera* L. corresponded to geographical dispersal [8]. Sabir et al. [12] analyzed of 17 indigenous cultivars and concluded that the relationship between genotypes was highly dependent on the geographic origin of the cultivars. However, our results obtained by using Jaccard's similarity index and ISSR markers, as well as the previously reported findings by application the Jaccard's similarity index based on RAPD markers and Euclidean distance based on ampelographic traits [13] indicate that the observed genetic diversity of cultivated and wild grape materials sampled in Azerbaijan is not geographically structured, by contrast to results of Maia et al. [7] performed

on Italian grape cultivars. These findings supported by paleontological evidence and historical information suggest that Azerbaijan is one of the centers of *V. vinifera* L. diversity, and Azerbaijan aborigine cultivars from regions near the Caspian Sea were originated from the local wild forms in this area.

Relationship between ISSR markers and biomorphological traits. Our studies revealed the correlation between two assessed biomorphological traits and ISSR alleles (table 2). The 2100 bp allele in UBC811 locus was found only in Sarigila tezyetishan, Ag uzum, Ghara shani, Absheron gizil uzumu, Sikhghara, Absheron kechimamasi cultivars and in wild accessions №32, №36 and №45. The average time of vegetation for these genotypes was 135.29 days. However, the average time of vegetation for the rest grapevine genotypes that had other sizes of alleles for UBC811 locus was 160.8 days which makes it possible to use detected 2100 bp allele of UBC811 locus as a genetic marker for identification of early ripening grapevine accessions.

Moreover, a correlation between 2500 bp allele of UBC810 locus and the weight of 100 berries is also observed. Thus, in Sarigila gezyetishan, Absheron kishmishi, Sikhghara, Iri salkhim, Ag uzum, Absheron kechimamasi and Sarigila ortayetishan cultivars which observed allele in 2500 bp size for UBC810 locus average of the weight of 100 berries were higher (110.7 g) than in other 52 grapevine accessions (an average of the weight of 100 berries was 37.96 g). However, to introduce these two loci as common genetic markers, additional studies are required.

Table 2

Genetic relationship between biomorphological traits and ISSR markers

Traits	ISSR markers	№.of genotypes	Mean	SD	F	T-test
Time of vegetation	UBC811	9	135.29	2.8	3.57	0.018 *
		51	160.8	5.87		
Weight of 100 berries	UBC810	8	110.7	0.89	4.18	0.03 *
		52	68.8	3.69		

* significant at 0.05 (P-value < 0.05)

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AZƏRBAYCANIN MƏDƏNİ VƏ YABANI ÜZÜM NÜMUNƏLƏRİNİN AMPELOQRAFİK VƏ MOLEKULAR MÜXTƏLİFLİKLƏRİNİN MÜQAYİSƏLİ TƏHLİLİ

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Azərbaycanın Xəzəryanı bölgələrinə məxsus mədəni və yabanı üzüm genotiplərinin filogenetik əlaqələrini öyrənmək məqsədi ilə nümunələrin ampeloqrafik göstəriciləri ilə ISSR markerləri əsasında aşkarlanmış genetik müxtəliflikləri müqayisə edilmişdir. Tədqiq olunan üzüm nümunələrinin fenotip və genotip səviyyələrində aşkar olunmuş genetik müxtəliflikləri onların coğrafi paylanmasına uyğun olmamışdır. Azərbaycanın öyrənilən üzüm populyasiyaları sırasında genetik müxtəlifliyin ən yüksək səviyyəsi Abşeron mənşəli mədəni üzüm populyasiyasına mənsub olmuşdur. Ampeloqrafik əlamətlərinə görə yabanı üzüm populyasiyası mədəni üzüm populyasiyasından ayrılmış olsa da, molekular səviyyədə mədəni və yabanı üzüm genofondları arasında genetik oxşarlıq müəyyən edilmişdir. Yabanı və mədəni üzüm nümunələri arasında genetik oxşarlığın aşkar edilməsi Azərbaycanın mədəni üzüm populyasiyasının bilavasitə, yerli yabanı üzüm nümunələrindən əmələ gəldiyini və bu coğrafi regionun *V.vinifera*-nın mümkün ilkin mənşə mərkəzlərindən biri olduğunu göstərir.

Açar sözlər: *Vitis spp.*, ampeloqrafiya, genetik məsafə, genetik oxşarlıq, ISSR markerləri

**СРАВНИТЕЛЬНАЯ ОЦЕНКА АМПЕЛОГРАФИЧЕСКОГО И МОЛЕКУЛЯРНОГО РАЗНООБРАЗИЯ
КУЛЬТУРНЫХ И ДИКИХ ОБРАЗЦОВ ВИНОГРАДА АЗЕРБАЙДЖАНА****С.Дж.Салаева, Дж.М.Оджаги, А.Т.Мамедов, И.А.Шахмурадов**

С целью выявления филогенетических связей между культурными и дикими виноградными образцами из прикаспийских регионов Азербайджана была проведена сравнительная оценка данных ампелографических признаков и ISSR маркеров. Генетическое разнообразие исследуемых образцов винограда, выявленное на фенотипическом и генотипическом уровнях не соответствовало их географическому распределению. Среди изученных виноградных популяций Азербайджана наиболее высоким уровнем генетического разнообразия отличилась культурная популяция Абшеронского происхождения. На основе ампелографических признаков дикая популяция винограда полностью обособилась от культурной, тогда как на молекулярном уровне культурные и дикие генофонды винограда оказались генетически сходными. Выявление генетического сходства между дикими и культурными виноградными образцами позволяет отметить, что культурные виноградные популяции Азербайджана произошли непосредственно от местных диких форм и этот географический регион является одним из возможных первичных центров происхождения *V.vinifera*.

Ключевые слова: *Vitis* spp., ампелография, генетическое расстояние, генетическое сходство, ISSR маркеры