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COMPARATIVE STUDY OF GRAPE (*Vitis vinifera* L.) GENETIC RESOURCES

M.K. Musayev, Corresponding Member of ANAS Z.I. Akparov

Were obtained data on the genetic characteristics of local genetic resources of grapes. Among the Azerbaijan samples, two synonymous were identified. In the cultivars included as out-group, synonymous were not identified. No samples showing an identical profile but different geographic origins were discovered. An average of 8.7 alleles per locus was detected. The most informative locus (I) was identified in the VVS2 locus.

Keywords: grapes, genetic resources, wild samples and local varieties

Introduction

Azerbaijan is characterized by high genetical and morphological variability of grapes - wild (*Vitis vinifera* L. subsp. *sylvestris* (Gmel.) Hegi) and cultivated (*Vitis vinifera* L. subsp. *sativa* (DC.) Hegi) samples. Archeological, paleobotanical and historical sources confirm that grapevines were spread and cultivated since a long time. One-two million year old grape leave fossilized imprints have been found in the western sides of the Bozdag mountain (Goy-gol region) and 0.5-1.0 million 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 Goy-gol region. It contained grape seeds and fossilized wine sediments. This finding is related to the Bronze Age (end of second and beginning of first millennium B.C.) [1, 3, 6, 9, 12]. In the period of intensive development of viticulture in Azerbaijan (1970-80s), the grapevine assortment of vineyards was enriched with foreign varieties from Western Europe, USA, 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. However, only 200 of them have been collected and included in field collections [2, 8, 10].

In Azerbaijan, viticulture and wine-making developed during centuries, producing hundreds of grape varieties selected for various

purposes (table, wine, universal, seedless raisins). Local grape varieties are mainly grown in old vineyards located in ancient settlements and homesteads. Different training systems were also found, including highpergola and low bush cultivations. Many regions of Azerbaijan are rich in valuable local grapevine varieties which have not been explored yet. Nowadays, Azerbaijan grape germplasm is studied with the support of national, foreign and international scientific organizations. Scientific expeditions have been conducted in different Azerbaijan regions (Garabagh, Quba-Khachmaz, Ganja-Gazakh, Nakhichevan, Sheki-Zaqatala, Shirvan, Apsheiron, etc.). The main results obtained include the finding of some not yet characterized local genotypes and their collection in experimental vineyards for the ampelographic evaluation. Also, thanks to international cooperation, data was obtained on the genetic features of the local genetic resources of grapes.

SSR markers offer a quick and robust method for varietal identification that was proposed by Thomas and Scott (1993) [15] and then widely adopted for variety identification and pedigree reconstruction in *Vitis vinifera* L. The growing interest in understanding the origins and genetic diversity of grape germplasms made this technique become the most prevalently used markers to solve questions of homonymy, synonymy and kinship.

Material and methods

Thirty-eight autochthonous grapevine cultivars from Azerbaijan, randomly selected

among the local germplasm of Country, were taken into account for this study. Twenty-two European varieties were included as outgroup.

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.

The samples were genotyped by 10 SSR markers: VrZag62; VrZag79; VVMD5; VVMD7; VVMD27; VVMD28; VVMD21; VVMD24; VVMD25; VVS2 [7]. Multiplexed PCR amplifications were performed in 25 µl final volume reaction mixture following the method described in De Lorenzis et al. (2012) [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).

In order to estimate the genetic diversity the different germplasms, the SSR data was used to determine the number of different alleles (Na), effective number of alleles (Ne), Shannon's Information Index (I), observed (Ho) and expected heterozygosity (He) per each germplasm. These data were performed by GenAlEx 6.5 software [13].

The association among the different germplasms were investigated based on the Principal Coordinate Analysis (PCoA) [14] approach, used to capture the correlation between genotypes. The PCoA analysis was carried out by GenAlEx 6.5 software, starting to the SSR correlation matrix.

Results and discussion

Sixty *V. vinifera* L. cultivars from Azerbaijan and 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 accession geographic origin are listed in Table. A total of 58 unique genotypes were detected. Among the Azerbaijan samples, two synonymous were identified. In the cultivars included as outgroup, synonymous

were not identified. No samples showing an identical profile but different geographic origins were discovered. An average of 8.7 alleles per locus was detected. The higher number of alleles was detected for Azerbaijani cultivars (10.2) and for VVMD28 locus of Azerbaijani samples (12.0). The number of different alleles ranged between 2.574 (VVMD21 locus of European cultivars) and 7.744 (VVMD28 of European cultivars). The most informative locus (I) was identified in the VVS2 locus of Azerbaijani samples. The mean value of Ho revealed by the analysis was high (0.800), ranging from 0.787 (Azerbaijan) to 0.814 (Europe). The He values were very similar to Ho values, showing a mean value of 0.777 and range from 0.751 (Europe) to 0.803 (Azerbaijan). Despite the number of analyzed accessions, number of analyzed loci and provenience of samples, the descriptive statistics reported in this study reflected the genetic diversity highlighted in previously works, such as Laucou et al. (2011) [7], where data about 2,836 SSR single profiles obtained by 20 SSR loci were described.

The correlation among cultivars was investigated by PCoA. The multivariate 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). The first two principal coordinates (PCo) accounted for 15.73 and 6.35% of the total variability. The distribution, along the PCo1 differentiated the samples into two main clusters: i) Azerbaijani group, where the most parts of samples coming from Azerbaijan were grouped; ii) European group, clustering the cultivars included ad outgroup. Among Azerbaijani germplasm, the group of samples more related to European 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 [4].

The West-East gradient, following the grapevine migration from the first domestication centre, clearly identified in the analysis performed by Myles et al. (2011) [11] was confir-

med. This differentiation of Azerbaijani samples could be due to the different usage of grapes. Indeed, the Azerbaijan cultivars are mostly table grape, because starting from 10th century AD, when the Azerbaijani people started to accept the Muslim religion, the population started to select table grapes instead of wine grapes.

Conclusions

The exploration of grapevine genetic resources by SSR (Simple Sequence Repeat) markers from Azerbaijan, considered one of the primary center of domestication of *V. vinifera* subsp. *sativa*, in order to identify the local genetic resources and understand the relationship

with the germplasm, nowadays, cultivated in Europe.

This work confirmed the usefulness of highly polymorphic SSR markers to improve information on genetic diversity and genetic relationship among cultivated germplasm growing in different geographical areas, i.e. Azerbaijan and Europe. The purpose of this study was to provide information about the genetic diversity of European and Azerbaijani varieties and the relationship among these different winegrowing areas. According to our results, a clear differentiation between Azerbaijani and European germplasms was identified.

Table

Genetic diversity of Azerbaijani and European cultivars revealed by analysis of 10 SSR loci.

Population	Locus	N ^a	Na ^b	Ne ^c	I ^d	Ho ^e	He ^f
Azerbaijan	VrZag62	38	10.0	6.926	2.087	0.789	0.856
	VrZag79	38	10.0	6.224	2.020	0.711	0.839
	VVMD5	38	9.0	4.011	1.755	0.711	0.751
	VVMD7	38	11.0	5.619	1.981	0.842	0.822
	VVMD21	38	8.0	3.871	1.615	0.868	0.742
	VVMD24	38	8.0	5.824	1.882	0.765	0.828
	VVMD25	38	11.0	7.627	2.190	0.867	0.869
	VVMD27	38	9.0	3.018	1.540	0.553	0.669
	VVMD28	38	12.0	4.821	1.928	0.921	0.793
	VVS2	38	14.0	7.424	2.258	0.842	0.865
	Total	38	10.2	5.536	1.926	0.787	0.803
Europe	VrZag62	22	6.0	3.681	1.537	0.773	0.728
	VrZag79	22	9.0	5.975	1.964	0.909	0.833
	VVMD5	22	7.0	5.438	1.797	0.909	0.816
	VVMD7	22	8.0	5.762	1.900	0.864	0.826
	VVMD21	22	6.0	2.574	1.231	0.727	0.612
	VVMD24	22	7.0	3.695	1.542	0.727	0.729
	VVMD25	22	5.0	3.546	1.400	0.773	0.718
	VVMD27	22	7.0	3.612	1.531	0.818	0.723
	VVMD28	22	9.0	7.744	2.102	1.000	0.871
	VVS2	22	8.0	2.907	1.452	0.636	0.656
	Total	22	7.2	4.493	1.646	0.814	0.751
Total		60	8.7	5.015	1.786	0.800	0.777

^a Number of samples; ^b Number of different alleles; ^c Number of effective alleles; ^d Shannon's Information Index; ^e Observed heterozygosity; ^f Expected heterozygosity.

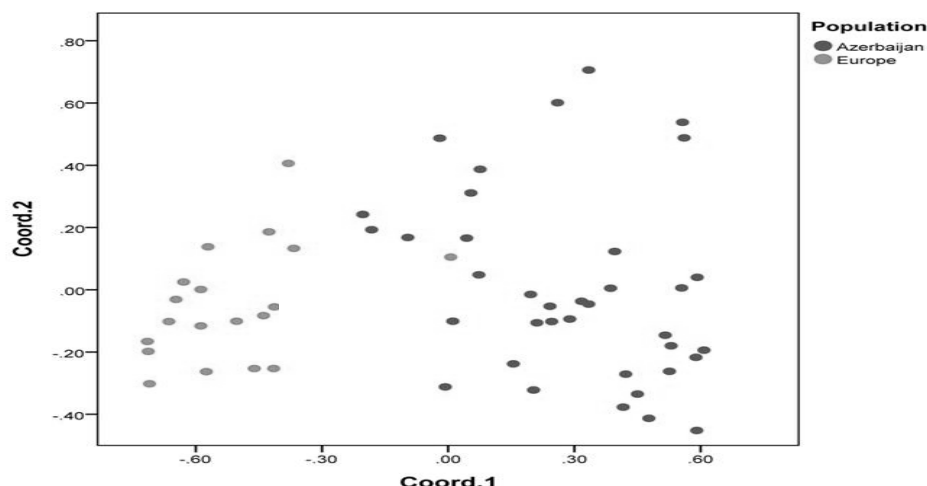


Figure. Plot of the first two principal coordinates of PCoA detected by 10 SSR data for 60 grapevine samples coming from Azerbaijan and Europe

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Genetic Resources Institute of ANAS
mirza.musayev@yahoo.com

ÜZÜMÜN (*Vitis vinifera* L.) GENETİK EHTİYATLARININ MÜQAYISƏLİ TƏDQIQI**M.K. Musayev, Z.İ. Əkrərov**

Üzümün yerli genetik ehtiyatlarının genetik xüsusiyyətləri haqqında məlumatlar əldə edilmişdir. Azərbaycan nümunələri arasında iki sinonim müəyyən edilmişdir. Üst qrup kimi daxil edilən sortlarda sinonimlər müəyyən edilməyib. Eyni profili göstərən, lakin fərqli coğrafi mənşəyi göstərən nümunələr aşkar edilmədi. Hər bir lokus üçün orta hesabla 8.7 allel aşkar edilmişdir. Ən informativ lokus (I) VVS2 lokusunda müəyyən edilmişdir.

Açar sözlər: üzüm, genetik ehtiyatlar, yabanı nümunələr və yerli sortlar

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ГЕНЕТИЧЕСКИХ РЕСУРСОВ ВИНОГРАДА (*Vitis vinifera* L.)**М.К. Мусаев, З.И. Акпаров**

Были получены данные о генетических характеристиках местных генетических ресурсов винограда. Среди азербайджанских образцов выявлено два синонима. В сортах, включенных в состав чужой группы, синонимов не выявлено. Образцов, показывающих идентичный профиль, но различное географическое происхождение, обнаружено не было. В среднем было обнаружено 8,7 аллелей на локус. Наиболее информативный локус (I) был идентифицирован в локусе VVS2.

Ключевые слова: виноград, генетические ресурсы, дикие образцы и местные сорта