International Journal of Minor Fruits, Medicinal and Aromatic Plants. Vol. 5 (1) : 07-10, June 2019

Results of the study of grape resources

Mirza Musayev

Head of Department of Fruit Crops,
Genetic Resources Institute of the Azerbaijan, National Academy of Sciences,
155, Azadliq Ave., AZ1106, Baku, Azerbaijan,

Email: mirza.musayev@yahoo.com

Received : 14.02.19 ; Revised : 10.04.19 ; Accepted : 30.04.19

ABSTRACT

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 origin were discovered. An average of 8.7 alleles per locus was detected. The most informative locus (I) was identified in the VVS2 locus.

***Keywords*:** Relationship, genetic resources, wild grapes and local varieties.

INTRODUCTION

Azerbaijan is considered as being a primary or secondary center of origin, domestication and diversity of many fruits including grapevine (Musayev and Huseynova, 2016). There is 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. 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 (Salimov and Musayev 2007, Salimov *et al*., 2008).

In Azerbaijan, viticulture and wine-making developed during centuries, producing hundreds of grape varieties selected for different 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 are 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, Apsheron, *etc*.). Aim of the work was 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 to understand the relationship with the germplasm, nowadays, cultivated in Europe.

SSR markers offer a quick and robust method for varietal identification that was proposed by Thomas and Scott (1993) and then widely adopted for variety identification and pedigree reconstruction in *Vitis vinifera* L.

MATERIAL AND METHODS

Plant materials

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 out-group.

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

Table 1: Genetic diversity of Azerbaijani and European cultivars revealed by analysis of 10 SSR loci. (Thomas and Scott, 1993).

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.

(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 ìl final volume reaction mixture following the method described in De Lorenzis *et al.* (2012). 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 of the different germplasms, the SSR data were 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 were

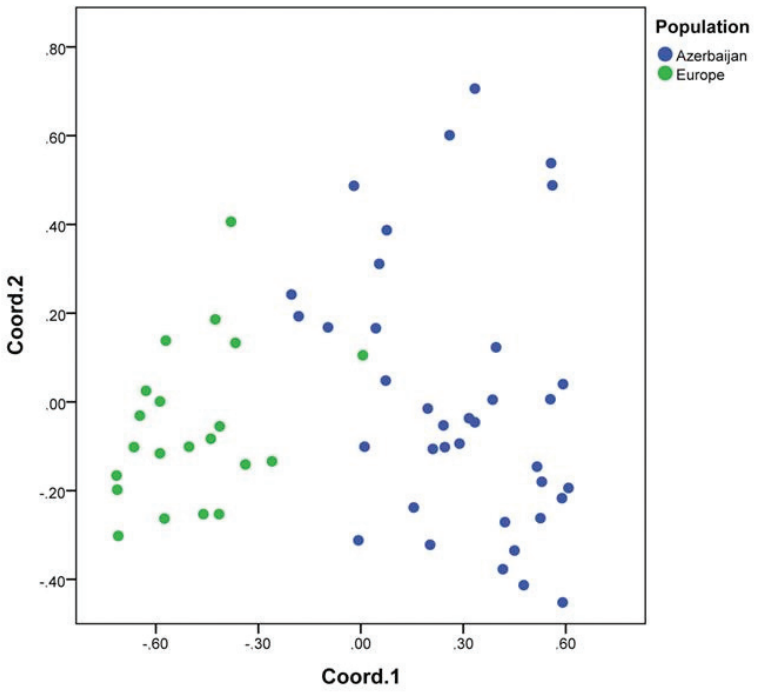

Figure 1: Plot of the first two principal coordinates of PCoA detected by 10 SSR data for 60 grapevine samples coming from Azerbaijan and Europe.

noted. These data were performed by GenAlEx 6.5 software (Peakall and Smouse, 2006).

The association among the different germplasms was investigated based on the Principal Coordinate Analysis (PCoA; Price *et al*., 2006) 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 1. A total of 58 unique genotypes were detected. 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 origin 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), where data about 2,836 SSR single profiles obtained by 20 SSR loci were descripted.

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 1). 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 part of samples coming from Azerbaijan were grouped; ii) European group, clustering the cultivars included ad out-group. 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 (Bacilieri *et al*., 2013).

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

CONCLUSION

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 wine growing areas. According to the results, a clear differentiation between Azerbaijani and European germplasms was identified.

REFERENCES :

Bacilieri, R.; Lacombe, T.; Le Cunff, L.; Di Vecchi-Staraz, M.; Laucou, V.; Genna, B.; Péros, J. P.; This, P.; Boursiquot, J. M.; 2013. Genetic structure in cultivated grapevines is linked to geography and human selection. *BMC Plant Bio*., **13,** 25.

De Lorenzis, G.; Imazio, S.; Biagini, B.; Failla, O.and Scienza, A.; 2012. Pedigree reconstruction of the Italian grapevine Aglianico (*Vitis vinifera* L.) from Campania. *Mol. Biotech.,* doi:10.1007/s12033-012-9605-9.

Laucou, V.; Lacombe, T.; Dechesne, F.; Siret, R.; Bruno, J. P.; Dessup, M.; Dessup, T.; Ortigosa, P.; Parra, P.; Roux, C.; Santoni, S.; Vares, D.; Peros, J. P.; Boursiquot, J. M. and This, P.; 2011. High throughput analysis of grape genetic diversity as a tool for germplasm collection management. *Theor. Appl. Genet.,* **122**:1233-45.

Musayev Mirza and Huseynova Taravat. 2016. Collection, study and use crop wild relatives of fruit plants in Azerbaijan. *International Journal of Minor Fruits, Medicinal and Aromatic Plants,* **2** (2):6-10.

Myles, S.; Boyko, A. R.; Owens, C. L.; Brown, P. J.; Grassi, F.; Aradhya, M. K.; Prins, B.; Reynolds, A.; Chia, J. M.; Ware, D.; Bustamante, C. D. and Buckler, E.S.; 2011. Genetic Structure and domestication history of the grape. *PNAS* **108:** 3530-35.

Peakall, R. and Smouse, P. E.; 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Eco. Notes* **6**, 288-295.

Price, A. L.; Patterson, N. J; Plenge, R. M.; Weinblatt, M. E.; Shadick, N. A. and Reich, D.; 2006. Principal components analysis corrects for stratification in genome-wide association studies. *Nat. Genet*. **38**, 904-09.

Salimov, V. S., Musayev M. K.; 2007. Grapevine genetic resources of Azerbaijan. Internet sources: www.vitis.ru/pdf/rs11.pdf (Russ)

Salimov, V. S., Quliyev, V. M. and Musayev, M. K.; 2008. Research of grapevine genetic resources in the Republic of Azerbaijan. *News Nat. Techn. Sci.,* **4**, 51-57 (Azeri).

Thomas, M.R. and Scott, N.S. 1993. Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). *Theoretical and Applied Genetics,* **86** (8): 985-90