Evaluation of DNA polymorphism between cultivated and wild grape varieties of Azerbaijan, in the case of the Shirvan-Shahı grape variety

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Archaeological excavations, paleobotanical studies, ampelographic data and written historical sources prove that the territory of Azerbaijan is one of the cultural centers of viticulture. Vavilov's research made it possible to establish that the South Caucasus is one of the world centers for the formation of many plants, including grapes. He believed that the Karabakh zone is the main center for species formation in the South Caucasus. In this study, it was aimed to reveal genetic diversity among grape samples originated from Azerbaijan by RAPD-based genotyping analysis. The minimum similarity between Shirvan-Shahı samples and wild grape samples is 50 percent. Among all studied genotypes, the highest genetic similarity was observed between VS6 and VVSH varieties with a similarity index of 0.954. Shirvan-Shahı grape varieties were taken from 6 different locations. VS6 and VVSH3 cultivars as well as VS6 and VVSH1 cultivars were also genetically very close with a similarity index of 0.912 and 0.902, respectively. VS6 and VVSH3 cultivars as well as VS6 and VVSH1 cultivars were also genetically very close with a similarity index of 0.912 and 0.902, respectively. Thus, the conducted studies confirm the hypothesis put forward by Negrul regarding the origin of Shirvan-Shahı from this ancestor and made it possible to confirm the genetic closeness of the studied forms.

Keywords: *V. vinifera* L. subsp. *sylvestris (C.C.Gmel), Shirvan-Shahi, RAPD*

INTRODUCTION

Archaeological excavations, paleobotanical studies, ampelographic data and written historical sources prove that the territory of Azerbaijan is one of the cultural centers of viticulture. Vavilov's research made it possible to determine that the South Caucasus is one of the world centers for the formation of many plants, including grapes. According to Vavilov, the main formation center in the South Caucasus is the Karabakh zone. Until now, wild forms of grapes grow on the banks of Kura and in the Karabakh zone. Many grape varieties cultivated in Azerbaijan have come from geographical areas where both table and technical grapes are endemic (Vavilov 1926; Vavilov 1931; Zhukovskii **)**.

Wild grapes have been distributed on the territory of Azerbaijan since ancient times. The germplasm of the wild grape *V. vinifera* L. subsp. sylvestris (C.C.Gmel.) is a potential source of unique alleles to improve both wine and table grapes for more efficient and sustainable grape production. In the South Caucasus region, the wild vine grows on numerous species of deciduous trees and shrubs in forests (Ocete et al., 2018).

Caucasian wild grapes are used in medicine, agriculture (pollination of female varieties) and food (male flowers for flavoring wines in Azerbaijan and unripe fruits (verjuice) in marinades and special sauces) (Maghradze et al., 2015). Genetic studies, including the distribution of haplotypes based on plastid DNA sequences, show a high level of variability in wild grapes (*V. vinifera* subsp. *sylvestris*) from the Greater Caucasus region (Pipia et al., 2012).

One of the famous indigenous grape varieties is *V. vinifera* L. Shirvan-Shahı. The Shirvan-Shahi variety has rich technological parameters and opportunities for creating a raw material base and increasing the export potential for the production of national wine brands in the country. It was grown only in the Shirvan region of the Araz plain of Azerbaijan, in the Kura-Aran subzone - in the Kurdamir region. The product of the variety is a valuable source for the purchase of high-quality dessert wines of the Cahors type. It was used in the preparation of dessert wine "Kurdamir" (Garagozov et al., 2004). At the beginning of the 20th century, the population of the Shirvan-Shahı variety, which

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occupied an area of more than 500 hectares in the Kurdamir region of Azerbaijan, was completely destroyed (Sadigova et al, 2021).

According to A.M.Negrul (1968), the Shirvan-Shahı variety originates from a wild form growing in the Tugai forests along the Kura River as a result of a bud mutation. In the future, it was propagated vegetatively in this form.

The purpose of this study was to identify the degree of genetic relationship between samples of the Shirvan-Shahı variety cultivated in its homeland in Kurdamir and its supposed wild ancestor, historically growing in forests along the Kura River. The evaluation of genetic diversity was enriched by the development of molecular marker technology. Molecular marker analysis is a powerful tool for grouping genetically closely related genotypes and sorting genetically distinct parents for a breeding program (Adhikari et al., 2017). The random amplified polymorphic DNA (RAPD) technique is based on the amplification and analysis of random regions of the genome of the organism in question, without requiring prior knowledge of the structure or sequence data of the grapevine. RAPDs are generated by polymerase chain reaction (PCR) on genomic DNA samples using randomly designed decamer-oligonucleotides as primers, yielding multiple amplified fragments of different sizes (corresponding to different loci), which can be separated and run on a standard agarose gel. In this study, it was aimed to reveal genetic diversity among grape samples originated from Azerbaijan by RAPD-based genotyping analysis.

MATERIALS AND METHODS

Plant material: 21 samples of grapes were taken as objects of study, of which 16 varieties of the genus *V. vinifera* and 6 varieties of *Vinifera* subsp. *sylvestris* originating from Azerbaijan. The grape samples and characteristics used in this study are listed in Table 1. Branches of grapes collected during field analyzes were used in *in vivo* propagation protocols (Garagozov et al., 2004).

gDNA isolation: 100 mg fresh leaves of grape samples were used in sodium dodecyl sulfate-based gDNA isolation protocol. The common protocol was followed as reported by Niu et al. (2008). Plant leaves were homogenized with 700 µL lysis buffer (containing 1% PVP) by using a sterile mortar and pestle. RNaseA treatment was carried out after the lysis step by adding 5 µL RNaseA of 10 mg/mL (Thermo, USA) to the homogenized samples. Samples (100-150 mg) were immediately transferred to a 1.5 ml microfuge tube containing 700 µl of preheated (60-65°C) sodium dodecyl sulfate (SDS) buffer. The solution was centrifuged at 15,000 rpm for 2 minutes, then cold chloroform was added at an available volume and the resultant solution was again centrifuged at 13,500 rpm for 3 minutes. To settle nucleic acids, cold isopropanol was added at about 60% of the available volume and centrifuged at 13,500 rpm for 3 minutes. Then the resultant precipitate was washed through with 500 µL 70% ethanol, and incubated at -20ºC overnight. A final concentration was centrifuged at 13,500 rpm for 5 minutes. The solution was precipitated with isopropanol, the precipitate was washed through 70% ethanol and dissolved in 50 µL distilled water. The quality and quantity of gDNA molecules were checked by agarose gels of 0.8% (70 V for 45 min) and a spectrophotometer (Thermo, USA), respectively.

RAPD fingerprinting: RAPD primers (Table 2) developed by Operon Technologies (Eurofins, France) were used in RAPD assays. PCR mixtures were prepared in a reaction volume of 25 µL containing 100 ng template DNA, 1 X PCR buffer, 3 mM $MgCl_2$, 0.4 mM of dNTPs, 20 pmol primer, 1 U *Taq* DNA polymerase (Promega, USA). The cycling conditions were as follows: pre-denaturation at 94ºC for 5 min; 35 cycles of 94ºC for 1 min, 38ºC or 45ºC for 1 min and 72ºC for 2 min; and final extension at 72ºC for 5 min. PCR bands were separated on agarose gels of 1.5% for 80 V for 1 hour, and then bands were visualized under a UV transilluminator (Hi-Media, India). Each experiment set was replicated at least three times.

Statistical analysis: RAPD markers were scored as '1' or '0' according to their presence or absence. Nei-Li's coefficient was used in order to obtain the similarity matrix (Nei and Li, 1979). The dendrogram was constructed by using UPGMA (unweighted pair-group method with arithmetic average) algorithm via MVSP 3.1 software (Kovach, UK).

RESULTS AND DISCUSSION

In experiments, 1-2 ocellar specimens 10-15 cm in size were used to obtain a primary explant. After sterilization of the samples, the lower parts of the shoots were cut off. The sterilized samples after 24-hour auxin stimulation were placed in a growth chamber in 200-300 ml containers filled with distilled water (Abdullaeva, 2017). Leaves were observed after 2 weeks. The conditions in the explant growing chamber were controlled and maintained: illumination 2000-3000 lux, temperature 24-26ºC and photoperiod 16/8 hours. After 3 weeks the leaves had developed enough to isolate the gDNA in all samples (Fig. 1).



Fig. 1. Grape samples in the growing cabin (after 2 weeks). Mikrotest mit-250.

gDNA molecules with high quality ($\Delta_{260/280}$=1.7-2.0) and quantity (x≥500 ng/µL) were obtained in SDS-based DNA isolation protocol and then they were diluted as 25 ng/µL in order to be used in PCR analysis. (Fig. 2).

In RAPD analysis, 6 primers yielded no bands or bands in only some of the samples whereas OPA13, OPA02, OPG13, and OPM02 primers gave bands from each sample's genomes (Table 2, Fig. 3). Totally 40 bands were obtained and 85% of these bands were polymorphic amplicons. The minimum and maximum band numbers were 9 (OPA02) and 11 (OPM02), respectively. RP and PIC values were in the ranges of 2.54-4.42 and 0.39-0.49, respectively.

The mean RP and PIC values were calculated as 3.34 and 0.43, respectively. The RAPD dendrogram showed two subclusters (Fig. 4). *V. vinifera* and *V. vinifera* subsp. *sylvestris* were grouped according to the branches of the dendrogram. The most genetically similar and dissimilar individuals were VSSH6 with BC6 (94.5%) and BB Kara_Urza with BC2 (45.8%) respectively (Table 3). The mean genetic similarity for RAPD analysis was 71.25%.

Table 1. Grape samples and characteristics used in this study.

No	Sample	Species	Usage	Origin	Latitude	Longitude
1	VS2	*V. vinifera subsp. sylvestris*	Universal	Kur riverside	40,069602	48,653625
2	VS3	*V. vinifera subsp. sylvestris*	Universal	Kur riverside	40,068929	48,653689
3	VS4	*V. vinifera subsp. sylvestris*	Universal	Kur riverside	40,065822	48,652038
4	VS5	*V. vinifera subsp. sylvestris*	Universal	Kur riverside	40,085317	48,675597
5	VS6	*V. vinifera subsp. sylvestris*	Universal	Kur riverside	40,029055	48,946719
6	VVSH1	*V. vinifera* ssp. *vinifera*	Technical	Kürdemir.	40,280436	48,199564
7	VVSH2	*V. vinifera* ssp. *vinifera*	Technical	Kürdemir.	40,281476	48,197355
8	VVSH3	*V. vinifera* ssp. *vinifera*	Technical	Kürdemir.	40,029122	48,948062
9	VVSH4	*V. vinifera* ssp. *vinifera*	Technical	Kürdemir.	40,358583	49,822779
10	VVSH5	*V. vinifera* ssp. *vinifera*	Technical	Kürdemir.	40,285297	48,188742
11	VVSH6	*V. vinifera* ssp. *vinifera*	Technical	Kürdemir.	40,275517	48,196045
12	Vv_Alashanı	*V. vinifera* ssp. *vinifera*	Table	Abşeron	40,530921	49,877444
13	Vv_Khındovnu	*V. vinifera* ssp. *vinifera*	Technial	Karabakh	39,931118	48,896182
14	Vv_Qara_Kurdashı	*V. vinifera* ssp. *vinifera*	Table	Kur riverside	39,691069	47,313129
15	Vv_Qara_Lınkını	*V. vinifera* ssp. *vinifera*	Technical	Karabakh fizuli	39,538563	47,15092
16	Vv_Qara_Urza	*V. vinifera* ssp. *vinifera*	Universal	Kur river side	40,057631	48,436573
17	Vv_Shıamahı_Merendısı	*V. vinifera* ssp. *vinifera*	Table	Shamakhi	40,58831	48,670047
18	Vv_Sıx_Qara	*V. vinifera* ssp. *vinifera*	Technical	Azerbaijan	40,452443	48,775038
19	Vv_Kecımemesı	*V. vinifera* ssp. *vinifera*	Technical	Azerbaijan	40,320322	49,123964
20	Vv_Arayatlı	*V. vinifera* ssp. *vinifera*	Technical	Azerbaijan	39,481564	47,377442
21	Vv_Medrese	*V. vinifera* ssp. *vinifera*	Technical	Karabakh	40,611569	48,560997

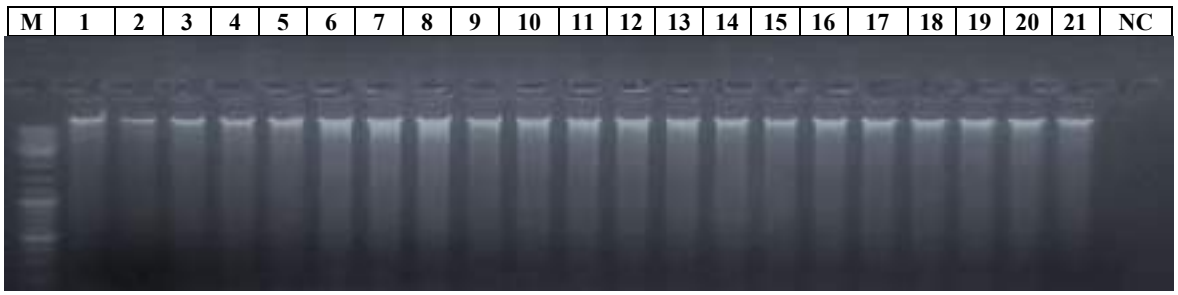

Fig. 2. Result from agarose gel electrophoresis analysis of 21 grape samples. M: 1 kb DNA ladder (Thermo, USA).

Table 2. RAPD primers used in this study. + indicates yield for all grape samples; - means no yield in PCR. * and ** mean that those primers were used in RAPD assays, respectively. nc: not calculated.

Primer	Sequence	Results	Total bands	Polymorphic bands	RP value	PIC value
OPA01**	CAGGCCCTTC	-	0	0	nc	nc
OPB14**	TCCGCTCTGG	-	0	0	nc	nc
OPC05**	GATGACCGCC	-	0	0	nc	nc
OPG16**	AGCGTCCTCC	-	0	0	nc	nc
OPM05**	GGGAACGTGT	-	0	0	nc	nc
OPM08**	TCTGTTCCCC	-	0	0	nc	nc
OPA13**	CAGCACCCAC	+	10	7	4.42	0.44
OPA02**	TGCCGAGCTG	+	9	8	2.54	0.39
OPG13**	CTCTCCGCCA	+	10	10	2.41	0.49
OPM02**	ACAACGCCTC	+	11	9	4	0.42
		Mean			**3,34**	**0,43**

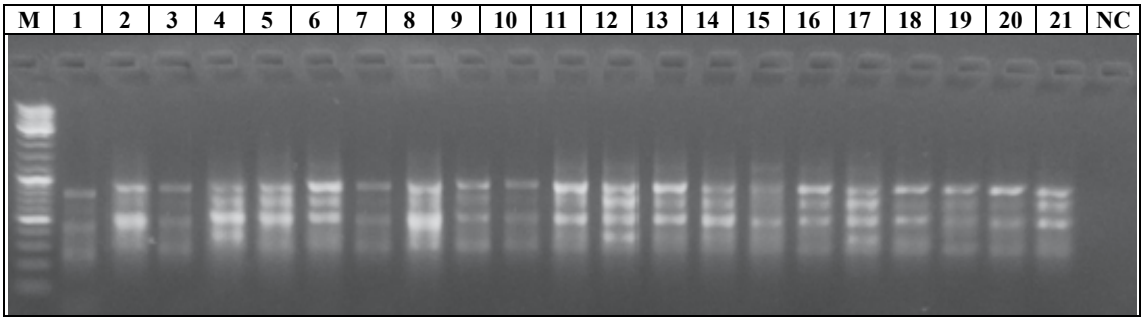

Fig. 3. OPG13 RAPD PCR profiles in grape samples. M: 1 kb DNA ladder (Thermo, USA), NC: no template control. Please follow the numbers given in Table 1.

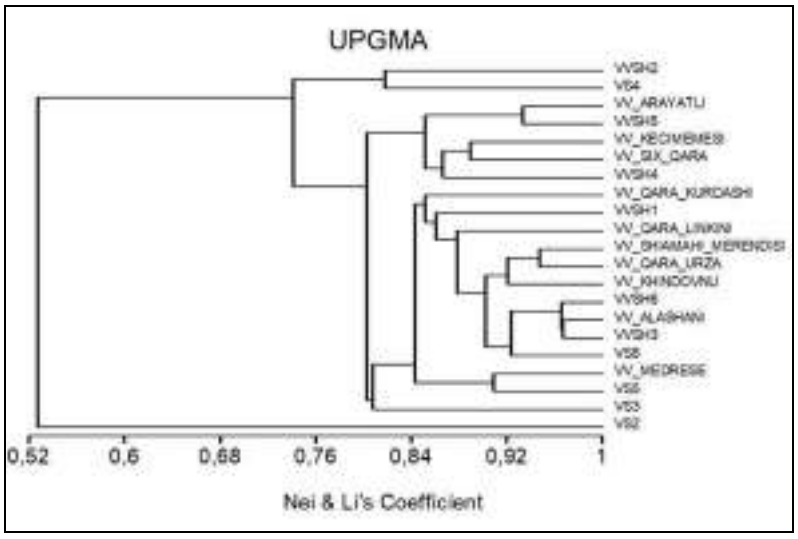

Fig. 4. RAPD dendrogram obtained from MVSP software.

It can be seen from the dendrogram that samples 1 to 5 form a separate cluster that is relatively separated from other samples. The similarity in this cluster ranges from 0.512 to 0.852, indicating a moderate to high degree of similarity between these samples (Fig. 4). These specimens belong to *V. vinifera subsp. sylvestris* taken from various nearby areas (around the Kura River). Wild grape - *V. vinifera* L. subsp. *sylvestris* (C.C.Gmel.) grows in many regions of Azerbaijan from 12 m below sea level (near the Kura River) to 2000 m above sea level (Gusar region) (Musaev et al ., 2013).

The minimum similarity between Shirvan-Shahı samples and wild grape samples is 50 percent. Among all studied genotypes, the highest genetic similarity was observed between VS6 and VVSH varieties with a similarity index of 0.954. Shirvan-Shahı grape varieties were taken from 6 different locations. VS6 and VVSH3 cultivars as well as VS6 and VVSH1 cultivars were also genetically very close with a similarity index of 0.912 and 0.902, respectively. VS6 and VVSH3 cultivars as well as VS6 and VVSH1 cultivars were also genetically very close with a similarity index of 0.912 and 0.902, respectively.

Thus, the conducted studies confirm the hypothesis put forward by Negrul regarding the origin of Shirvan-Shahı from this ancestor and made it possible to confirm the genetic closeness of the studied forms (Negrul., 1968).

The average genetic similarity among *V. vinifera* samples was higher than the average similarity index among wild genotypes. Studies demonstrate that *V. Vinifera* L. subsp. *sylvestris (*C.C. Gmel.*)* grape samples are more distant than cultivated grape samples. However, the obtained results leave open the debatable question about the origin of wild forms, namely, whether they are uncultivated representatives of *Vitis Vinifera* or, in this case, are a unique form of wild grapes.

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Şirvan-Şahı üzüm sortu nümunəsində Azərbaycanın mədəni və yabanı üzüm sortları arasında DNT polimorfizminin qiymətləndirilməsi

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Arxeoloji qazıntılar, paleobotanika tədqiqatları, ampeloqrafik məlumatlar və yazılı tarixi mənbələr Azərbaycan ərazisinin üzümçülüyün mədəniyyət mərkəzlərindən biri olduğunu sübut edir. Vavilovun tədqiqatları Cənubi Qafqazın bir çox bitkilərin, o cümlədən üzümün formalaşması üçün dünya mərkəzlərindən biri olduğunu müəyyən etməyə imkan verdi. O hesab edirdi ki, Qarabağ zonası Cənubi Qafqazda növlərin formalaşması üçün əsas mərkəzdir. Bu tədqiqatda RAPD əsaslı genotip analizi ilə Azərbaycan mənşəli üzüm nümunələri arasında genetik müxtəlifliyin aşkarlanması məqsədi daşımışdır. Şirvan-Şahi nümunələri ilə yabanı üzüm nümunələri arasında minimum oxşarlıq 50% təşkil etmişdir. Bütün tədqiq edilmiş genotiplər arasında ən yüksək genetik oxşarlıq 0,954 oxşarlıq indeksi ilə VS6 və VVSH sortları arasında müşahidə edilmişdir. VS6 və VVSH3 sortları, həmçinin VS6 və VVSH1 sortları da müvafiq olaraq 0,912 və 0,902 oxşarlıq indeksi ilə genetik cəhətdən çox yaxın idi. VS6 və VVSH3 sortları, həmçinin VS6 və VVSH1 sortları da müvafiq olaraq 0,912 və 0,902 oxşarlıq indeksi ilə genetik cəhətdən çox yaxın idi. Beləliklə, aparılan tədqiqatlar Negrul.-un irəli sürdüyü fərziyyəni təsdiqləyir ki, bu da Şirvan-Şahinin bu əcdaddan mənşəyi haqqında məlumat vermiş və tədqiq olunan formaların genetik yaxınlığını təsdiq etməyə imkan vermişdir.

Açar sözlər: *V. vinifera* L. subsp. *sylvestris (CC Gmel), Şirvan-Şahi, RAPD*