Evaluation of DNA Polymorphism among Cultivated and Wild Grapevine Accessions from Azerbaijan

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Abstract: To estimate genetic relationships among 31 cultivated and 34 wild grape accessions originating from regions near the Caspian Sea in the Azerbaijan Republic, RAPD analysis was performed with 27 decamer primers selected from a total of 55 primers. The most discriminating primers were OPC-16, OPF-18 and OPA-17, which showed the highest values of genetic diversity (0.927, 0.914 and 0.909, respectively). The lowest values of diversity pertained to the markers OPA-1 (0.615) and V-20 (0.624). The cluster analysis representing genetic similarity among all selected samples divided the genotypes into nine separate groups at similarity index 0.508. Within the studied Azerbaijan grape populations the highest genetic diversity belonged to the population of cultivated samples originating from the Absheron peninsula, with a diversity index 0.852 and the next ranks were assigned to the wild populations originating from Nabran and Guba regions, with a diversity index 0.824 and 0.793, respectively. The lowest diversity was observed within Davachi individuals, with a diversity index 0.765. The wild population from Azerbaijan was molecularly similar to the cultivated gene pool from this area. This result supported the hypothesis that the southwest of the Caspian Sea is a region where grape was brought into culture. During the analysis a special band was observed which could be used in identifying wild and cultivated grape accessions with high or low resveratrol content. The results of this work clearly indicated that the RAPD analysis can be used to estimate genotypic similarities, genetic diversity and for clustering cultivated and wild grape accessions.

Keywords: Azerbaijan wild and cultivated grape; genetic diversity; RAPD markers; resveratrol; similarity index

The grape is unique: not only it is a major global horticultural crop but also it has ancient historical connections with the development of human culture. In the family *Vitaceae* it is the genus *Vitis* that is of major agronomic importance. It consists of ~ 60 mutually crossable species that exist almost exclusively in the northern hemisphere (This *et al.* 2006). Currently, *Vitis vinifera* is among the most important plant species cultivated on an area of about 7.9 million ha and there exist more than 10 000 grape cultivars worldwide, with annual production of approximately 58 million tons (FAO 2004).

Modern crops are relatively recent descendents of their wild ancestors, and although the genetic distance between the cultivated and wild varieties is increased by the effects of human selection and the population bottleneck accompanying domestication, it is still necessary to type as many variable loci as possible to conduct meaningful studies of agricultural origins.

Although wild grapes were probably present in many places in Europe during the Neolithic period, archaeological and historical evidences suggest that primo-domestication occurred in the Near East.

The earliest evidence of wine production was found in Iran at the Hajji Firuz Tepe site in the northern Zagros Mountains circa 7400–7000 BP. Seeds of domesticated grapes dated from ~8000 BP were also found in Georgia and in Turkey. However, events leading to the domestication of this crop species are still an open issue (Zohary & Spiegel-Roy 1975; Zohary & Hopf 2000; McGovern 2004; Doulaty Baneh *et al.* 2007).

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 (Suleymanov & Mammadov 1982). Archaeological materials are said to be the main sources to enlighten the socio-economic life of Azerbaijani people, moreover for the learning of the vine-growing history in Azerbaijan. Archaeological heirloom contains samples of dried bunches of grapes found under the soil as well as in jars preserving the shape and size (Sharifov 2005). Remainders of grape plants and seeds possessing traces of cultivation and dating back to the 5th–6th millennium BC were found near the Agstafa region in Shomutepa in 1962, which indicated that there has been 7 thousand years of vine cultivation in Azerbaijan (Negrul 1973). Natural conditions of the Azerbaijan Republic, in other words, ecological conditions of 10 natural economic zones of the country such as Apsheron; Guba-Khachmaz; Sheki-Zagatala; Mughan-Salyan; Lankaran-Astara mountain zones; Garabagh-Mil; Gyanja-Gazakh mountain downhill zone; Nakhichevan Autonomous Republic; Mountain Karabagh; Shirvan zones are very suitable for grape development. Investigations and surveys revealed that more than 600 indigenous and introduced grape varieties are widespread in Azerbaijan (Amanov 1998).

Traditional methods of describing grapevine varieties based on the plant vegetative and reproductive traits (ampelography) have contributed greatly to establishing the identity and relationships among *V. vinifera* cultivars (Krimbas 1943; Negrul 1946; Galet 1979; Boursiquot *et al.* 1987). Nevertheless, ampelographic traits are often plastic, with a great genotype × environment components interaction rendering them less useful in classifying closely related cultivars. In recent years, molecular markers have proved to be a valuable tool for genetic studies and cultivar characterization. The random amplified polymorphic DNA (RAPD) technique has several distinct advantages: the cost per reaction is low, only a small amount of plant material is required for DNA extraction, and the method does not require any prior knowledge of the sequence of the genome (Karatash & Sabit Agaoglu 2008). Theoretically, the polymorphisms revealed by the RAPD markers are mainly due to nucleotide substitutions or insertions/deletions (Ren *et al.* 2003). The RAPD technique has been successfully used for the identification of grapevines (Vidal *et al.* 1999; Regner *et al.* 2000; Ulanovsky *et al.* 2002; Pinto-Carnide *et al.* 2003; Benjak *et al.* 2005; Kocsis *et al.* 2005).

Resveratrol (3, 5, 4'-trihydroxystilbene) is a stilbene phytoalexin and a natural component of grape plant tissues (Woo *et al.* 2007). Resveratrol has been found to inhibit the proliferation of a variety of human cancer cell lines, including those from breast, prostate, stomach, colon, pancreatic, and thyroid cancers when added to cells cultured outside the body. In addition, resveratrol has a protective effect on the myocardial function (Sato *et al.* 2000), protects the isolated heart of rat from ischaemia, increases nitric oxide synthesis, has relaxant effects on isolated porcine coronary arteries and inhibits the factor expression in vascular cells. Since the measurement of resveratrol content needs much costs and time, the identifying markers for distinguishing genotypes with maximum resveratrol are very important.

The objective of this study was to estimate the genetic diversity between grape varieties and wild relatives originating from the Near-Caspian zone of Azerbaijan, using RAPD markers.

MATERIAL AND METHODS

Plant material. A total of 65 accessions, comprising 31 local table grape varieties (*Vitis vinifera* ssp. *sativa*) and 34 wild types (*Vitis vinifera* ssp. *sylvestris)* originating from regions near the Caspian Sea of the Azerbaijan Republic, were analyzed. All plant materials were sampled from the grape collection of the Genetic Resources Institute of the Azerbaijan National Academy of Sciences and from the Azerbaijan Scientific Research Institute of Viticulture and Wine-making. Local and wild accessions used in this study and their origins are listed in Table 1.

DNA extraction. Total plant DNA was isolated from green tissues of cuttings using a modified

Table 1. Names and origins of grape accessions investigated in the study

Cultivated samples			Wild samples	
No.	names	origin	No.	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	Giziluzum	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

version of the CTAB protocol described by Doyle and Doyle (1990). The tissues were scrapped and 1 g was ground using a mortar and pestle under liquid nitrogen. The powder was then suspended in 5 ml of extraction buffer 1 [0.2M Tris-HCl (pH 8.0), 0.07M EDTA (pH 8.0), 2M NaCl, 0.02M $Na_2S_2O_5$]. 500 µl of this suspension was transferred into an Eppendorf tube and 450 µl of buffer 2 containing 2% cetyl trimethyl ammonium bromide (CTAB), 1.4M NaCl, 0.02M EDTA (pH 8.0) and 0.1 Tris-HCl (pH 8.0) was added. After thorough agitation using a vortex the mix was incubated for an hour

at 65°C in the water bath (mixed several times during incubation), then centrifuged at 10 000 g at 4°C for 5 min. The supernatant was transferred into a new tube with 450 µl chloroform/octanol (24:1). After mixing and centrifugation at 10 000 g at 4°C for 15 min 300 µl of supernatant was mixed with 300 µl isopropanol (–20°C) and 150 µl of 10M CH_3COONH_4, incubated at –20°C during 25 min and then precipitated at 10 000 g at 4°C for 10 min. The precipitated DNA was washed in 750 µl of 70% ethanol. The pellet was dried in Speed-Vac, dissolved in 200 µl 0.1× TE (1mM Tris-HCl pH=8.0, 0.1mM EDTA pH=8.0) at 4°C overnight.

RAPD amplification. The primers were chosen from literature records based on their ability to reveal high levels of polymorphism. The sequences of the 27 decamer primers used in the study are shown in Table 2. Each 20 µl PCR reaction was carried out using 6 ng of template DNA in 20 µl of total reaction volume containing 10mM Tris-HCl (pH 8.3), 50mM KCl, 0.01% gelatine, 2.5mM $MgCl_2$, 0.2mM each of the dNTP's, 0.2mM primer and 0.5 unit *Taq* DNA polymerase. Amplification was performed using a Gene Amp 9700 thermocycler (Invitrogen, Carlsbad, USA) according to the following programme: 2 min at 94°C, then 45 cycles of 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. Amplification products were separated by electrophoresis using 2% agarose gels with 1× TAE buffer (40mM Tris-acetate and 1mM EDTA pH 8.0) at 3 V/cm during 4 h. After running, the gels were stained with ethidium bromide (0.05%) and visualised on a UV transilluminator (Invitrogen, Carlsbad, USA). A 100 bp ladder was used as a molecular size standard. Each sample was processed at least twice to confirm genotype reproducibility.

Data analysis. Photographs were performed with a Gel Doc 1000 camera (Molecular Analyst program of Bio-Rad). Data were recorded as presence (1) or absence (0) of each amplification band, to construct the band matrix. After the identification of bands and patterns among genotypes a genetic diversity index was calculated based on the following formula (Nei 1973) for each marker:

$$H = 1 - \Sigma P_i^2$$

where:

H – genetic diversity index

P_i – frequency of the i^{th} pattern

A dendrogram representing genetic similarity was constructed according to Jaccard's similarity index following the UPGMA (unweighted pair group method with arithmetic averages) from the genetic distance matrix, as a graphic representation of the relationships among samples. These analyses were performed using the NTSYSpc 2.11V software package (Rohlf 2004). A genetic distance (Nei 1978) between the investigated Azerbaijan grape populations was calculated on the basis of Nei's genetic distance index using the POPGENE 1.1V program (Yeh & Boyle 1997).

RESULTS AND DISCUSSION

Degree of polymorphism. In order to investigate the genetic diversity of the grape accessions originating from regions near the Caspian Sea in the Azerbaijan Republic, 55 RAPD primers were utilized. An initial screening with 55 primers was carried out on four varieties, Uzunsov gilali ag shani, Iri gilali ag shani, Ag shani, Ag shani (clone). These varieties were chosen for their ampelographic coincidences in some characters. As a consequence, 27 informative primers were selected due to their ability to produce polymorphic and unambiguous markers between these varieties. By 27 primers 522 bands were revealed in total, nevertheless, 464 of them were resolvable and reproducible. The size of amplification products selected for statistical analysis ranged from 250 to 3000 base pairs. 157 bands (33.84%) were common in all accessions and 307 bands (66.16%) exhibited polymorphism. These data were compared with some other reports performed in grape; Kocsis *et al*. (2005) observed 120 polymorphic fragments with 28 RAPD markers for characterization of 12 Carpathian Basin grape cultivars, Aras *et al*. (2005) detected 49.5% of polymorphic bands among Buzgulu grape ecotypes *(Vitis vinifera* L.) grown in different regions of Turkey, while Ergul *et al*. (2002) determined 110 polymorphic bands with 22 RAPD markers to identify 17 Turkish grape cultivars. In our study the number of bands for each primer ranged from 8 (OPA-1) to 28 (PHA-09), with an average of 17.18. Among the employed primers, the primers OPC-16 (Figure 1), P-166 and OPF-18 with 18, 17, and 16 bands had the highest number of polymorphic bands, respectively, while the primer OPA-1 with 5 bands had the lowest number of polymorphic bands. The

Table 2. List of the primers, their sequences and genetic parameters used for the analysis

Primer	Sequence (5'-3')	Total bands	Number of analyzed bands	Number of polymorphic bands	Percent of polymorphism	Genetic diversity index
PHA-02	GGTCCTCAGG	18	17	12	70.59	0.852
PHA-09	GGTAGCAGTC	32	28	11	39.28	0.763
OPA-1	CAGGCCGTTC	10	8	5	62.5	0.615
OPA-10	CTGATCGCAG	19	17	11	64.7	0.747
OPA-03	AGTCAGCCAC	20	14	10	71.43	0.775
OPA-09	GGGTAACGCC	14	13	10	76.92	0.719
OPA-11	CAATGGCCGT	16	13	8	61.53	0.674
OPA-15	TTCCGAACCC	23	20	10	50.00	0.761
OPA-17	GACCGCTTGT	18	14	13	92.85	0.909
OPO-02	ACGTAGCGTC	19	18	10	55.55	0.889
OPO-03	CTGTTGCTAC	24	21	12	57.14	0.884
OPO-04	AAGTCCGCTC	28	20	13	65.00	0.866
OPO-07	CAGCACTGAC	14	14	8	57.14	0.763
OPO-19	GGTGCACGTT	18	16	11	68.75	0.794
OPC-16	CACACTCCAG	24	24	18	75.00	0.927
OPF-02	GGACACCACT	29	19	12	63.16	0.812
OPF-16	GAAGTACTGG	21	15	10	66.67	0.728
OPF-18	TTCCCGGGTT	17	17	16	94.12	0.914
OPF-19	CCTCTAGACC	17	17	13	76.47	0.731
V-20	CAGACTGGTC	12	12	9	75.00	0.624
X-18	GACTAGGTGG	14	14	8	57.14	0.641
P-123	GGGATTCGAC	19	18	8	44.44	0.721
P-232	CCGCTTGTTG	17	17	15	88.23	0.854
P-166	GTGACGGACT	24	24	17	70.83	0.885
B-06	TGCTCTGCCC	22	21	15	71.43	0.894
B-07	GGTGACGCAG	19	19	11	57.89	0.811
UBC-792	CAACCCACAC	14	14	11	78.57	0.785
Total		522	464	307	–	–
Mean		19.33	17.18	11.37	67.12	79.03

mean number of polymorphic bands was 11.37 for each individual primer. The highest percentage of polymorphic bands was detected with the primers OPF-18 (94.12%), OPA-17 (92.85%), and P-232 (88.23%), whereas the lowest percentage of polymorphic bands related to the primers PHA-09 (39.28%) and P-123 (44.44%). The average of the observed polymorphism per primer was 67.12%. The most discriminating primers were OPC-16, OPF-18 and OPA-17, which showed the highest values of genetic diversity (0.927, 0.914 and 0.909, respectively). The lowest values of diversity pertained to the markers OPA-1 (0.615) and V-20 (0.624). In total, the primers OPC-16 and OPF-18 with the highest number of polymorphic bands and the highest value of genetic diversity were recognized to be the most appropriate primers for studies related to genetic diversity of culti-

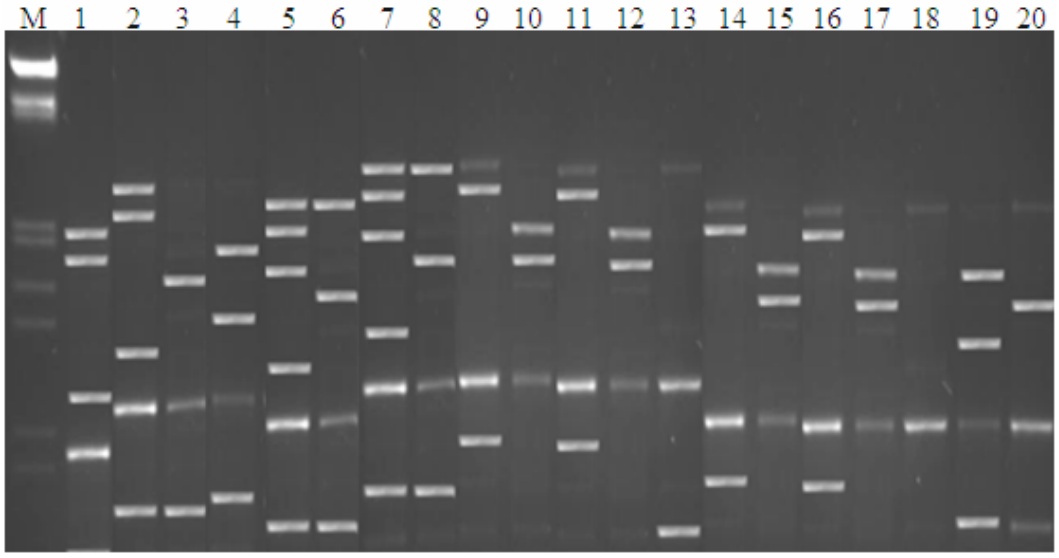

Figure 1. An example of electrophoregram obtained with primer OPC-16; the numbers indicate cultivars as listed in Table 1

vars and wild grape accessions. The next primers to be recommended are the primer P-166, which was among primers with the highest number of polymorphic bands and the primer OPA-17, which assigned the highest level of genetic diversity. All the 27 used primers could identify all genotypes. The suitability of the RAPD technique for genetic diversity studies and germplasm evaluations has been shown in many studies (Kocsis *et al*. 2005; Bodea *et al*. 2009; Maia *et al*. 2009). The data on genetic relationships among grape genotypes obtained from the RAPDs are in agreement with the data obtained using other markers. In view of the fact that the genotype identification is considered as one of the main goals of grape molecular studies, the capacity of RAPD primers used in this study in the identification of all samples is of great importance.

Among the bands reproduced by the primer OPF-18, one of the replicated DNA fragments (2300 bp) was observed only in genotypes with high resveratrol content. An example of electrophoregram obtained with primer OPF-18 is shown in Figure 2. The *t*-test statistically confirmed this relation. As a result, this primer can be used as

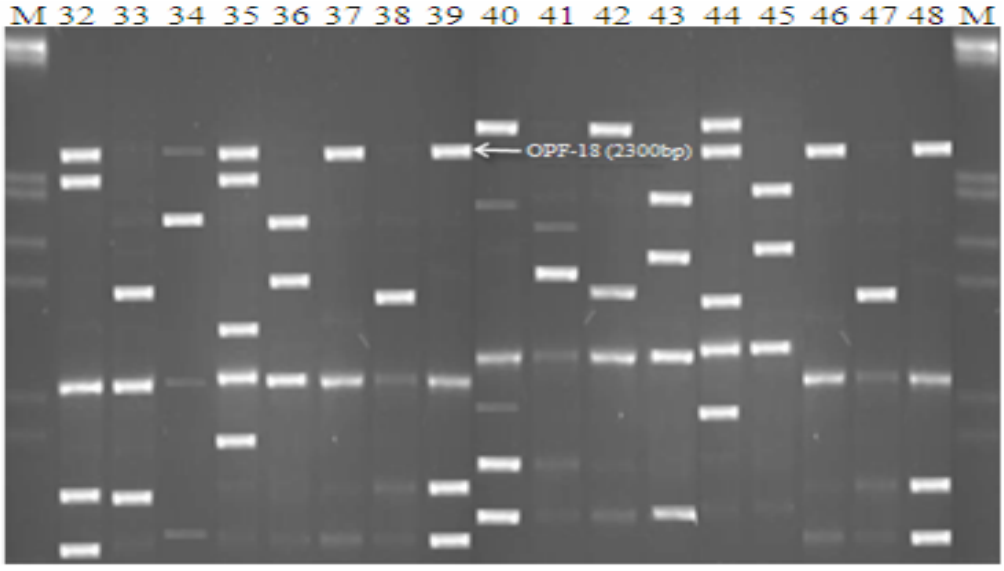

Figure 2. An example of electrophoregram obtained with primer OPF-18; the numbers indicate wild accessions as listed in Table 1

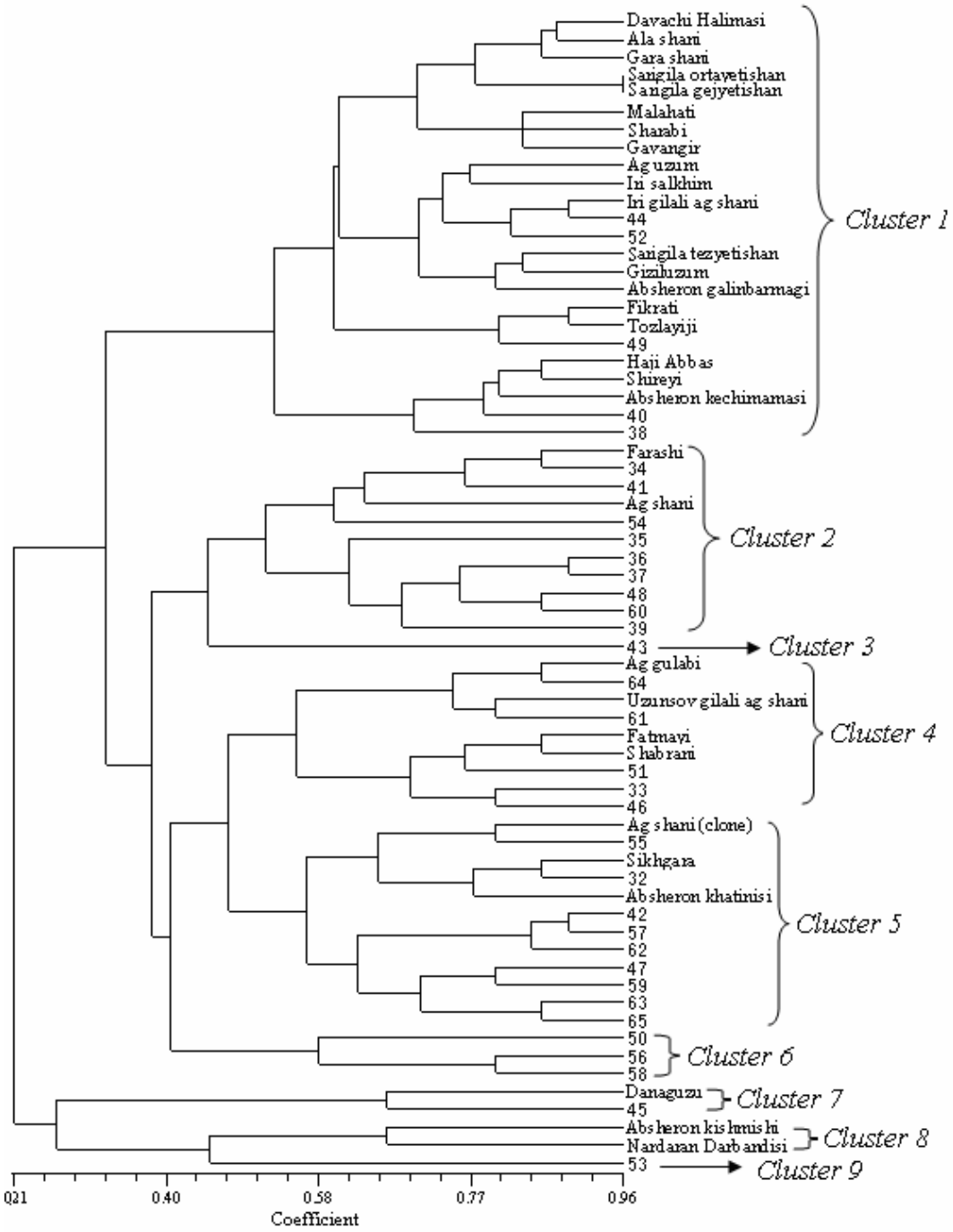

Figure 3. Dendrogram showing the genetic relationship among investigated Azerbaijan grapevines

SCAR marker to help quickly identify genotypes with high or low resveratrol content.

Genetic relationship and distance between the genotypes. A dendrogram representing genetic similarity among all selected samples is demonstrated in Figure 3. Among all investigated samples, the highest level of genetic similarity (0.959) was observed between the cultivated samples Sarigila ortayetishan and Sarigila gejyetishan. In contrast, the lowest level of genetic similarity (0.212) was detected between wild sample No. 53 sampled from Davachi region and the cultivated sample Ala shani belonging to the Absheron peninsula. An average genetic similarity among all studied samples was 0.597. According to the results obtained from the dendrogram, all the genotypes were divided into nine separate groups at a similarity index 0.508. The first group contained 24 genotypes, includ-

ing 19 cultivars, wild samples No. 44, 52, 40, 38 originating from Davachi region and wild sample No. 49 originating from Guba region. This cluster was assigned the highest number of genotypes, i.e. 36.92% of all genotypes, among the nine separated groups. As observed, 79.17% of the genotypes in this cluster are cultivated samples. Among the samples in this group, the cultivars Fikrati and Tozlayiji as well as the cultivated sample Iri gilali ag shani and wild sample No. 44 sampled in the Davachi region appeared very close genetically, with a similarity index 0.915 and 0.91, respectively. On the contrary, the cultivated sample Davachi halimasi and wild sample No.38 from Davachi region, with a similarity index 0.531, showed the lowest level of similarity. In the second group which included 11 genotypes, i.e. 16.92% of all genotypes, in addition to the two cultivated samples Farashi and Ag shani, 9 wild samples were present, including samples No. 34 and No. 35 originating from Nabran region, samples No. 41, 54, 36, 37, 48 and 39 from Davachi region, and samples No. 60 from Guba region. The highest level of genetic similarity among the samples in this group related to samples No. 36 and No. 37 from Davachi region, with a similarity index 0.918. The lowest level of genetic similarity belonged to the cultivated sample Farashi and wild sample No. 39 from Davachi region, with a similarity index 0.517. The third group included only one wild genotype (No. 43) sampled from Guba region and therefore this cultivar belongs to a separate group which indicates the genetic distance of this sample from the other investigated genotypes. The fourth group contained 9 genotypes, i.e. 13.85% of all genotypes; 4 of them were cultivars, namely Ag gulabi, Uzunsov gilali ag shani, Fatmayi and Shabrani, two were wild samples No. 64 and 33 from Nabran region and other three genotypes were wild samples No. 61, 51 and 46 from Guba region. Among these cultivars, the highest genetic similarity belonged to Ag gulabi and to wild sample No. 64 (similarity index 0.848) and lower genetic similarity was observed between the cultivated samples Fatmayi and Shabrani, with a similarity index 0.846. The lowest level of similarity related to the cultivated sample Ag gulabi and to wild sample No. 46 from Guba region (with a similarity index 0.551). The fifth group consisted of 12 genotypes, which made up 18.46% of all examined genotypes. In this cluster, most of the accessions were wild, besides only 3 cultivated samples, namely Ag shani (clone), Sikhgara and Absheron khatinisi. The wild genotypes grouped in this cluster, like in the second group, were sampled in different regions of Azerbaijan, so as wild samples No. 55 and 42 originated from Davachi, sample No. 32, 62, 63 and 65 belonged to Nabran region and three samples No. 57, 47 and 59 belonged to Guba region. The highest genetic similarity in this group was revealed between samples No. 42 and 57 (0.883), while the lowest genetic similarity was detected between the cultivated sample Ag shani (clone) and wild sample No. 65, with a similarity index 0.567. The sixth group included only three wild genotypes originating only from Davachi region. In this cluster, samples No. 56 and No. 58 acquired the highest genetic similarity (0.801), while cultivars No. 50 and 58, with a similarity index 0.582, had the highest genetic distance from each other. The seventh group included two genotypes Danagozu and wild genotype No. 45 originating from Davachi region. The value of similarity index for these two samples was 0.659. Although the eighth group included two cultivated samples, namely Absheron kishmishi and Nardaran Darbandisi, the genetic distance between these two cultivars was exactly the same as that between wild sample No. 45 and the cultivar Danagozu. The ninth group, like the third group, included only one wild sample No. 53 originating from Davachi, which shows the existence of genetic distance between this genotype and other investigated samples.

In some studies intraspecies genetic diversification of *V. vinifera* corresponded to geographical dispersal (Moncada *et al.* 2006). However, in the present study, the dendrogram generated using Jaccard's similarity index showed that the observed genetic diversity of cultivated and wild grape material sampled in Azerbaijan is not geographically structured, like the researches of Maia *et al.* (2009) performed on Italian grape cultivars.

Thus, the results of cluster analysis showed that the wild accessions originating from regions near the Caspian Sea in Azerbaijan were closely related to aborigine cultivars from this area and we can draw a tentative conclusion that these cultivated accessions originated from wild accessions of this area. Following these results, our suggestion is that the southwest of the Caspian Sea has the highest probability of being the geographical area within which wild grape was domesticated.

Determination of genetic distance among the populations. In order to determine the distance

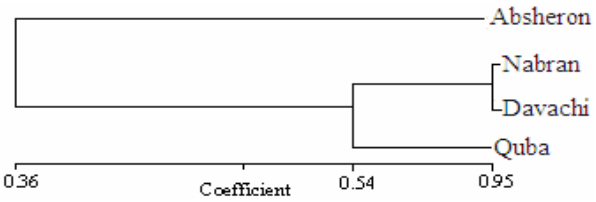

Figure 4. Dendrogram showing the genetic distance among studied grape populations

among the Azerbaijan grape populations under study, a dendrogram (Figure 4) was constructed based on Nei's genetic distance index (1978) and UPGMA method. As observed, the population of cultivated genotypes has the longest distance from the three investigated wild populations, which shows its genetic divergence from these wild populations. Moreover, among the wild populations, the shortest genetic distance pertained to the samples from Davachi and Nabran regions, which reveals their high similarity. The samples originating from Quba have a longer distance from these two populations.

Within the studied Azerbaijan grape populations the highest genetic diversity belonged to the population of cultivated samples originating from the Absheron peninsula, with a diversity index 0.852 and the next ranks were assigned to the wild populations originating from Nabran and Guba regions, with a diversity index 0.824 and 0.793, respectively. The lowest diversity was observed within Davachi individuals, with a diversity index 0.765. Perhaps the higher number of cultivated samples compared to wild samples originating from the three investigated regions is one of the causes of their higher genetic diversity. However, the observed genetic diversity of grape cultivars, especially remarkable diversity of cultivated samples originating from the Absheron peninsula, is very important for breeding programs; based on the cross-pollination nature of this plant, also using the observed potential of these cultivars it is possible to increase the heterozygosity level within the population by hybridization methods reaching the maximum value of heterosis. Although the number of wild accessions sampled in Davachi region was approximately twice the number of accessions sampled in Nabran and Quba regions, the observed genetic diversity of Nabran and Quba samples was higher in comparison with the Davachi samples. The existing diversity in wild samples is very significant as the domestication pressure on cultivated samples has caused considerable losses of many useful genes, such as those responsible for resistance to different types of diseases and environmental stresses (drought, salinity, cold, heat, etc). Therefore, wild samples are very valuable reservoirs of useful genes which can resolve many of our present and future problems.

Acknowledgements. We would like to thank Dr. S. Decroocq for modified version of the DNA extraction protocol. We are grateful to Dr. R. Eshghi and Dr. J. Ojaghi for their help in statistical analyses; to Dr. V. Salimov and Dr. M. Musayev for providing part of the wild and cultivated plant material. The authors also acknowledge valuable support from Dr. Z. Akparov (director of Genetic Resources Institute of Azerbaijan National Academy of Sciences) and Dr. A. Mammadov.

References

Amanov M.V. (1998): Local and Introduced Grape Grades of Azerbaijan Republic. Azerbaijan Ministry of Agriculture, Baku.

Aras S., Burgin P.J., Cansaran D., Solyemezoglu G. (2005): RAPD analysis of genetic relations between Buzgulu grape cultivars (*Vitis Vinifera*) grown in different parts of Turkey. Acta Biologica Cracoviensia, **47**: 77–82.

Benjak A., Ercisli S., Vokurka A., Maletić E., Pejić I. (2005): Genetic relationships among grapevine cultivars native to Croatia, Greece and Turkey. Vitis, **44**: **73–77**.

Bodea M., Pamfild D., Pop R., Pop I.F. (2009): Use of random amplified polymorphic DNA (RAPD) to study genetic diversity among Romanian local vine (*Vitis vinifera* L.) cultivars. Bulletin UASVM Horticulture, **66**: 1843–5394.

Boursiquot J.M., Faber M.P., Blachier O., Truel P. (1987): Computerization and statistical analysis of ampelographic data. Agronomie, **7**: 13–20.

Doulaty Baneh H., Mohammadi S.A., Labra M., Nazemieh A., De Mattia F., Mardi M. (2007): Chloroplast microsatellites markers to assess genetic diversity in wild and cultivated grapevines of Iran. Pakistan Journal of Biological Science, **10**: 1855–1859.

Doyle J.J., Doyle J.L. (1990): Isolation of plant DNA from fresh tissue. Focus, **12**: 13–15.

Ergul A., Marasali B., Agaoglu Y.S. (2002): Molecular discrimination and identification of some Turkish grape cultivars (*Vitis vinifera* L.) by RAPD markers. Vitis, **41**: 159–160.

FAO (2004): Food and Agriculture Organization of the United Nations International statistics. Crop Production by Country, Includes Fruit, Vegetables, Grape and Vine. FAO Year Book: Production. Available at http://www.fao.org/documents

Galet P. (1979): A Practical Ampelography-Grapevine Identification. Cornell University Press, Ithaca.

Karatash H., Sabit Agaoglu Y. (2008): Genetic diversity among Turkish local grape accessions (*Vitis vinifera* L.) using RAPD markers. Hereditas, **145**: 58–63.

Krimbas V. (1943): Greek Ampelography. Volume 1. Ministry of Agriculture, Athens.

Kocsis M., Jaromi L., Putnoky P., Kozma P., Borhidi A. (2005): Genetic diversity among twelve grape cultivars indigenous to the Carpathian Basin revealed by RAPD markers. Vitis, **44**: 87–91.

Maia S.H.Z., Mangolin C.A., Collet S.A.O., Machado M.F.P.S. (2009): Genetic diversity in somatic mutants of grape (*Vitis vinifera*) cultivar Italia based on random amplified polymorphic DNA. Genetics Molecular Research, **8**: 28–38.

McGovern P.E. (2004): Ancient Wine: the Search for the Origins of Viniculture. Princeton University Press, New Yersey.

Moncada X., Pelsy F., Merdinoglu D., Hinrichsen P. (2006): Genetic diversity and geographical dispersal in grapevine clones revealed by microsatellite markers. Genome, **49**: 1459–1472.

Negrul A.M. (1946): Origin and classification of cultured grape. In: Baranov A., Kai Y.F., Lazarevski M.A., Palibin T.V., Prosmoserdov N.N. (eds): The Ampelography of the USSR. Volume 1. Pischepromizdat, Moscow, 159–216.

Negrul A.M. (1973): Ampelography of Azerbaijan USSR. Azerbaijan State Publishing, Baku.

Nei M. (1973): Analysis of gene diversity in subdivided population. Proceedings of the National Academy of Sciences, **70**: 3321–3323.

Nei M. (1978): Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, **89**: 583–590.

Pinto-Carnide O., Martín J.P., Leal F., Castro I., Guedes-Pinto H., Ortiz J.M. (2003**):** Characterization of grapevine (*Vitis vinifera* L.) cultivars from northern Portugal using RAPD and microsatellite markers. Vitis, **42**: 23–25.

Regner F., Wiedeck E., Stadbauer A. (2000): Differentiation and identification of white riesling clones by genetic markers. Vitis, **39**: 103–107.

Ren F., Lu B.R., Li S., Huang J., Zhu Y. (2003): A comparative study of genetic relationships among the AA-genome *Oryza* spiecies using RAPD an SSR markers. Theoretical and Applied Genetics, **108**: 113–120.

Rholf F. (2004): NTSYSpc Version 2.1, Numerical Taxonomy and Multivariate Analysis System. Department of Ecology and Evolution, State University of New York.

Sato M., Cordis G.A., Maulik N., Das D.K. (2000): SAPKs regulation of ischemic preconditioning. American Journal of Physiology – Heart and Circulatory Physiology, **279**: 901–907.

Sharifov F.H. (2005): The Grape of Azerbaijan. Onder Neshriyyat, Baku.

Suleymanov J.S., Mammadov R.A. (1982): Viticulture. Maarif, Baku.

This P., Lacombe T., Thomas M.R. (2006): Historical origins and genetic diversity of wine grapes. Trends in Genetics, **22**: 511–519.

Ulanovsky S., Gogorcena Y., Toda F.M., Ortiz, J. M. (2002): Use of molecular markers in detection of synonymies and homonymies in grapevines (*Vitis vinifera* L.). Scientia Horticulturae, **92**: 241–254.

Vidal J.R., Coarer M., Defontaine A. (1999): Genetic relationships among grapevine cultivars grown in different French and Spanish regions based on RAPD markers. Euphytica, **109**: 161–172.

Woo S.Y., Kim D.H., Jun C.B., Kim Y.M., Haar E.V., Lee S.I., Hegg J.W., Bandhakavi S., Griffin T.J., Kim D.H. (2007): PRR_5, a novel component of mTOR complex 2, regulates platelet-derived growth factor receptor beta expression and signalling. Journal of Biological Chemistry, **282**: 25604–25612.

Yeh F.C., Boyle T.J.B. (1997): POPGENE Version 1.1. Department of Renewable Resources, University of Alberta, Edmonton.

Zohary D., Spiegel-Roy. (1975): Beginnings of fruit growing in the old world. Science, **187**: 319–327.

Zohary D., Hopf. (2000): Domestication of Plants in the Old World: The Origin and Spread of Cultivated Plants in West Asia, Europe and the Nile Valley. Oxford University Press, Oxford.

Received for publication February 17, 2010
Accepted after corrections June 6, 2010

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