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Genetic and biochemical differentiation in *Vitis vinifera* (Kabarcik) populations grown at different altitudes in Coruh Valley

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ABSTRACT. We examined genetic differences of four *Vitis vinifera* populations (A, B, C, D) including local Kabarcik cultivar grown along an altitude gradient of 800, 900, 1000, and 1150 m above sea level in the Coruh Valley (800 m: A population; 900 m: B population; 1000 m: C population; 1150 m: D population). Leaf samples were used for both RAPD and fatty acid analysis. A total of 60 individuals with 15 individuals per population were included in this study. RAPD analyses showed various band sizes, which ranged from 250 to 3000 bp. Mean polymorphic locus ratios were determined as 96.29% considering four populations. The highest percentage of polymorphic loci (97.8%) was produced by the highest altitude. Thirty-two different fatty acids were found; linoleic acid was the most common in all four populations. According to the dendograms obtained from statistical analyses of RAPD and fatty acid profiles the populations that were close to each other in terms of geographical distance also were similar genetically.

Key words: FAME; RAPD; *Vitis vinifera*; Genetic distance; Altitude

INTRODUCTION

Grape (*Vitis vinifera*) belongs to the Vitaceae family and is one of the oldest cultivated fruits in the world. Grapevines have been cultivated for about 5000 years (Zohary and Hope, 2000). The Eurasian grape (*Vitis vinifera* L.) is the most widely cultivated and economically important fruit crop in the world (Vivier and Pretorius, 2002). In *V. vinifera* L. around 6000 cultivars are estimated to exist (Alleweldt and Dettweiler, 1994), of which less than 400 are of commercial importance (Galet, 2000). It is of great economic importance, and its fruit is mostly used for transformation into valuable beverages such as wines and spirits. Each Anatolia region claims its own cultivars and wines.

During cultivation, populations of cultivated plants are subject to strong selective pressures, e.g., human determination of morphological, physiological and behavioral changes in the organisms (Otero-Arnaiz et al., 2005). This process has greatly modified the natural mating systems and dispersal mechanisms of plant species (Pickersgill, 1969), as well as their morphology, physiology and genetic structures (Doebley, 1989; Buckler et al., 2001; Hernández-Verdugo et al., 2001; Zeder et al., 2006). The genetic structures of cultivated plant populations have been shaped by many factors over the course of cultivation (Hernández-Verdugo et al., 2001; Oyama et al., 2006).

Many molecular genetic markers have been used in detecting population genetic structure since 1966. Among them, random amplified polymorphic DNA (RAPD) is easy to use. RAPD has become an increasingly popular tool in genetic studies of natural plant populations (Nybom and Bartish, 2000). RAPD markers developed during the last two decades have been successfully and widely utilized for taxonomic and systematic classification as well as phylogenic and genetic diversity studies of plants (Rath et al., 1998; Sun et al., 2005; Meimberg et al., 2006; Hug and Roger, 2007). The RAPD technique has several advantages over isozyme and other DNA markers, including speed, low cost, and the use of small amounts of plant material. It has therefore been widely used for estimating genetic diversity and relatedness in plant populations (Huff et al., 1993; Heum et al., 1994; Ge et al., 1999).

Genetic diversity of plant species can significantly change with the variation of altitudes that regulate ecological conditions in a particular plant habitat (Gail et al., 1998; Hsiao and Lee, 1999; George et al., 2001; Erich and Johann, 2003; Semang et al., 2003; Feng et al., 2004). Consequently, a change of genetic diversity in plant populations along an altitude gradient becomes an increasingly attractive subject both for theoretical interests and conservation practices because sometimes a relatively small change in altitude can cause a drastic change in environmental conditions, such as temperature and moisture (Heath and Williams, 1979; Baur and Raboud, 1988). Previous studies have shown that the genetic diversity of plant populations may increase (Yan et al., 1999), decrease (Gail et al., 1998; Zhao et al., 2001) or fluctuate to a large extent (Li et al., 1998), with an increase in altitude.

In the present study, we investigated the level and pattern of genetic variation at RAPD markers in four populations of *V. vinifera* (Kabarcik) along an altitude gradient ranging from 800 to 1150 m above sea level in the Erzurum.

MATERIAL AND METHODS

Plant material

Four cultivated (A, B, C, D) populations of *V. vinifera* (Kabarcik) were sampled along

an altitude gradient - 800, 900, 1000, and 1150 m above sea level - in the Coruh Valley (800 m: A population; 900 m: B population; 1000 m: C population; 1150 m: D population). A total of 60 individuals with 15 individuals per population were included in this study.

Extraction and analysis of FAME

Preparation and analysis of FAME from whole cell fatty acids from plant samples were performed according to the method described in the manufacturer manual (Sherlock Microbial Identification System version 4.5, MIDI Inc., Newark, DE, USA). Plant leaves were powdered after lyophilization in liquid nitrogen. Approximately 40 mg powdered leaf material from each sample was added to 1 mL 1.2 M NaOH in 50% aqueous methanol with 5 glass beads (3 mm in diameter) in a screw cap tube, then incubated at 100°C for 30 min in a water bath. After the saponified samples were cooled at room temperature for 25 min, they were acidified and methylated by adding 2 mL 54% 6 N HCl in 46% aqueous methanol and incubated at 80°C for 10 min in a water bath. After rapid cooling, methylated fatty acids were extracted with 1.25 mL 50% methyl-tert butyl ether (MTBE) in hexane. Each sample was mixed for 10 min and the bottom phase was removed with a Pasteur pipette. The top phase was washed with 3 mL 0.3 M NaOH. After mixing for 5 min, the top phase was then removed for analysis. Following the base wash step, the extract (FAME) was cleaned in anhydrous sodium sulfate and then transferred to a GC sample vial for analysis.

FAME were separated by gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA, USA) with a fused-silica capillary column (25 m by 0.2 mm) with cross-linked 5% phenyl methyl silicone. The operating parameters for the study were set and controlled automatically by a computer program. The chromatograms with peak retention times and areas were produced on the recording integrator and were electronically transferred to the computer for analysis, storage and report generation. Peak naming and column performance were achieved through the use of Eukary calibration standard mix (Microbial ID 1201-A) containing nC9-nC30 saturated and 2- and 3-hydroxy fatty acids. Cellular fatty acids were identified on the basis of equivalent chain length data. FAME profiles of each plant species tested were identified by comparing the commercial databases (Eukary) with the MIS software package.

DNA extraction and polymerase chain reaction (PCR) amplification

Genomic DNA was extracted from powdered plant materials using frozen nitrogen, as described by Lin et al. (2001).

RAPD

Samples were screened for RAPD variation using standard 10 base primers supplied by Operon. Thirty microliters of reaction cocktail was prepared as follows: 3.0 µL 10X buffer, 1.2 µL 10 mM dNTPs, 1.2 µL 25 mM magnesium chloride, 2.0 µL 5 µM primer, 0.4 µL 5 U *Taq* polymerase, 19.2 µL water, and 3.0 µL DNA sample (100 ng/µL). A total of 35 RAPD primers were tested and among them 10 primers producing reproducible banding patterns were selected. Therefore, the results are based on 10 primers. The thermal cycler (Eppendorf Company) was programmed for 2 min at 95°C; 2 cycles of 30 s at 95°C, 1 min at 37°C, 2 min at

72°C; 2 cycles of 30 s at 95°C, 1 min at 35°C, 2 min at 72°C; 41 cycles of 30 s at 94°C, 1 min at 35°C, 2 min at 72°C; followed by a final 5-min extension at 72°C, then brought down to 4°C.

Electrophoresis

The PCR products (27 µL) were mixed with 6X gel loading buffer (3 µL) and loaded onto an agarose (1.5%, w/v) gel electrophoresis in 0.5X TBE (Tris-borate-EDTA) buffer at 70 V for 150 min. The gel was stained in ethidium bromide solution (2 µL Etbr/100 mL 1X TBE buffer) for 40 min and visualized under UV in Bio Doc Image Analysis System with Uvisoft analysis package (Cambridge, UK).

Data analysis

Amplified DNA fragments were scored by the presence (1) or absence (0) for each DNA sample, which formed a matrix of the RAPD phenotypes. For FAME analysis, fatty acids of each plant species were scored as present (0.1-100%) or absent (0%). The genetic distance or similarity was determined by Jaccard (1908) similarity index. The pairwise genetic distances of the samples were used to construct a dendogram using the unweighted pair group method of arithmetic average (UPGMA).

RESULTS AND DISCUSSION

Results of the RAPD analysis are summarized in Table 1. In total, 132 bands were generated from 60 individuals from the 4 populations using 10 primers. The highest number of bands (23) was produced by OPB-8 and the lowest number bands (7) was produced by OPW-4. The size of the amplified fragments ranged from 250 to 3000 bp. The percentage of polymorphic loci across populations varied between 94.6 and 97.8%, with an average of 96.29%. The highest percentage of polymorphic loci, 97.8%, was produced by the D population.

Table 1. Primers employed with the number of RAPD markers obtained, their sequence, the size of the fragments, and the percentage of polymorphic markers (P) for each primer.

Primer code	Sequence 5'→3'	Total bands	A population	B population	C population	D population	Range of fragment size (bp)
OPA-4	AATCGGGCTG	16	12	16	10	15	250-3000
OPB- 8	GTCCACACGG	23	23	11	7	12	300-2500
OPB-10	CTGCTGGGAC	12	8	10	12	12	500-2700
OPH-17	CACTCTCCTC	15	13	4	10	15	500-2900
OPW-4	CAGAAGCGGA	7	7	5	7	6	400-1900
OPW-7	CTGGACGTCA	11	11	9	4	4	500-1700
OPW-11	CTGATGCGTG	12	3	12	5	2	700-2000
OPW-13	CACAGCGACA	15	7	12	15	4	400-2500
OPW-17	GTCCTGGGTT	12	5	11	3	12	600-3000
OPW-20	TGTGGCAGCA	9	3	7	2	9	500-1900
Total % polymorphism		132	95.83	96.90	94.66	97.80	

The 10 random primers generated a total of 92 RAPD bands in the A population. The size of the amplified fragments ranged from 600 to 2500 bp. OPB-8 gave the highest number of RAPD products, while OPW-11 and OPW-20 gave equally the lowest numbers. In total, 95.83% of the bands were polymorphic.

The 10 random primers generated a total of 97 RAPD bands in the B population. The size of the amplified fragments ranged from 250 to 3000 bp. Primers OPA-4 and OPH-17 gave the highest and lowest number of RAPD products, respectively. In total, 96.90% of the bands were polymorphic.

The 10 random primers generated a total of 75 RAPD bands in the C population. The size of the amplified fragments ranged from 400 to 2100 bp. Primers OPW-13 and OPW-20 gave the highest and lowest number of RAPD products, respectively. In total, 94.66% of the bands were polymorphic.

The 10 random primers generated a total of 91 RAPD bands in the D population. The size of the amplified fragments ranged from 250 to 3000 bp. Primers OPA-4, OPH-17 and OPW-11 gave the highest to lowest number of RAPD products, respectively, in descending order. In total, 97.8% of the bands were polymorphic. Banding patterns of the D population genotypes using the primer OPB-8 are illustrated in Figure 1. The dendrogram created from the RAPD markers grouped the 4 populations into three major clusters (Figure 2). Cluster 1 formed the A population, Cluster 2 formed the B population. Cluster 3 was divided into 2 subclusters and C and D populations formed a subcluster. The genetic distance between the population A and D (0.335) is high. The dendrogram clearly shows that each population is genetically differentiated from the other populations.

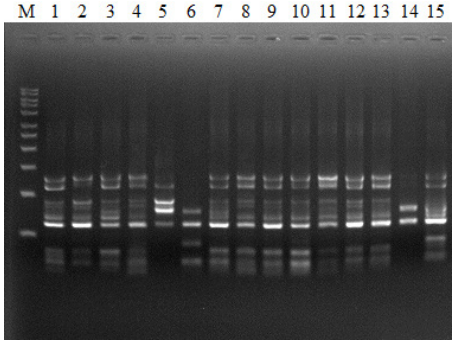

Figure 1. Amplification products generated from 15 individuals of D populations of *Vitis vinifera* (Kabarcık) using primer OPB-8. M = molecular marker.

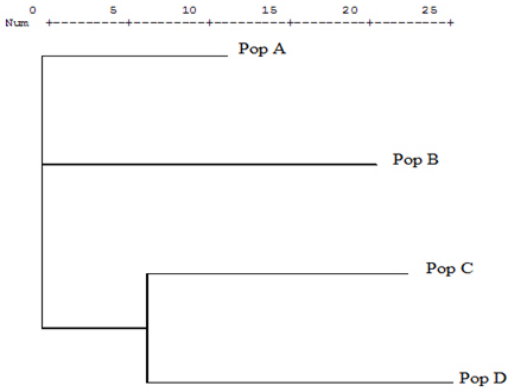

Figure 2. Dendrogram of genetic distances based on RAPD 4 populations of *Vitis vinifera* (Kabarcık).

RAPD analyses showed various band sizes, which ranged between 250 and 3000 bp. Mean polymorphic locus ratios were determined to be 96.29%. The highest percentage of polymorphic loci, 97.8%, was produced by the D population. High polymorphism explains the rich genetic resource of *V. vinifera* (Kabarcik).

The percent of fatty acid content of *V. vinifera (*Kabarcik) for the A population is presented in Table 2. As seen in the Table, the A population has 13 kinds of fatty acids and their fatty acid content ranged from 1.23% (14:0) to 78.78% (18:1:ω9c). All individuals had 16:0 and 18:1:ω9c content that ranged between 11.21- 26.96 and 33.37-78.78%, respectively. 14:0, 10:0 3 OH, 12:1:ω8c, 16:1:ω3c, 24:5:ω3, and Cis9,10epoxy 18:0 are rarely seen and found in low concentrations. Oleic (18:1; 33.37 to 78.78% within population) acid forms the bulk of the total fatty acids found. Biochemical similarity index amongst the individuals of the A population ranged between 0.222 and 1.000.

Table 2. Composition of fatty acids of the A population.

Fatty acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
14:0	-	-	-	-	-	-	-	-	-	-	-	-	1.23	-	-
10:0 3 OH	-	-	-	-	-	1.77	-	-	-	-	-	-	-	-	-
12:1:ω8c	-	-	-	-	-	1.54	-	-	-	-	-	-	-	-	-
16:0	26.96	11.39	15.27	20.69	19.43	14.18	14.32	14.68	21.22	11.28	17.07	12.71	11.21	15.09	19.62
16:1:ω3c	-	-	-	-	-	-	3.06	-	-	-	-	-	-	-	-
18:1 ω9c	33.37	64.53	63.65	64.93	41.67	60.11	69.01	64.45	78.78	46.20	65.12	46.64	58.04	75.04	68.12
21:1:ω7c	39.67	-	-	-	20.43	-	-	-	-	-	-	14.19	-	-	-
24:5:ω3	-	-	-	-	-	2.67	-	-	-	-	-	-	-	-	-
25:0 3 OH	-	-	-	-	18.47	-	-	-	-	-	-	-	-	-	-
26:0 3 OH	-	18.71	11.50	14.38	-	13.90	13.61	16.12	-	-	16.58	26.46	30.75	8.09	12.26
28:0	-	-	9.57	-	-	5.84	-	-	-	-	-	-	-	-	-
C25N alcohol	-	5.37	-	-	-	-	-	4.75	-	36.22	-	-	-	-	-
Cis9,10epoxy 18:0	-	-	-	-	-	-	-	-	-	6.30	-	-	-	-	-

The B population contains the highest fatty acids of all the populations. Table 3 represents the percent fatty acid content of the B population. The population contains 22 different fatty acids in a range of 1.03% (C21 primary alcohol) to 72.93% (18:1:ω9c). All individuals had 18:1:ω9c content that ranged between 30.28 and 72.93%. 16:1:ω3c, 16:1:ω9, 18:1:ω5c, 18:0, 19:1:ω7c, 20:0, 24:5:ω3, 28:0, ISO 17:1 G are rarely seen and found in low concentrations. ISO 17:1 AT9 and ISO 17:G are found only in three individuals in a range of 2.20-5.29%. Oleic (18:1; 2.17 to 72.38% within population) acid forms the bulk of the total fatty acids found. Biochemical similarity index amongst the individuals in the B population ranged between 0.111 and 1.000.

Table 4 represents the percent fatty acid content of the C population. The population contains 13 different fatty acids in a range of 0.96% (24:0 2 OH) to 76.74% (18:1:ω9c). All individuals had 16:0 and 18:1: w9c content that ranged between 11.93 and 29.19%, 45.81 and 76.74%, respectively. 16:1:ω3c, 18:0, 18:1:ω5c, 18:1:ω9t alcohol, 19:1:ω7c, 24:0 2 OH, 25: 0, and 28: 0 are rarely seen and found in low concentrations. Oleic (18:1; 1.28 to 76.74% within the population) acid forms the bulk of the total fatty acids found. 24:0 2 OH was found only in the C population. Biochemical similarity index amongst the individuals of the C population ranged between 0.222 and 1.000.

Table 5 represents the percent fatty acid content of the D population. The population contains 21 different fatty acids in a range of 1.76% (16:1:ω9) to 69.79% (18:1:ω9c). 16:1:ω3c, 16:1:ω9, 18:1:ω12c alcohol, 18:1:ω9t alcohol, 18:3:ω3c alcohol, 19:1:ω7c, 20:0,

Table 3. Composition of fatty acids of the B population.

Fatty acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
16:0	17.09	13.59	20.49	10.84	13.39	14.51	-	19.34	16.94	11.70	11.10	14.18	15.06	15.06	7.91
16:1:ω3c	-	2.29	-	-	2.28	-	-	-	-	-	-	2.54	-	-	-
16:1cis7DMA(ω9)	-	-	-	-	2.41	-	-	-	-	-	-	-	-	-	-
18:1:ω5c	-	-	-	-	-	2.20	-	-	-	-	-	-	-	-	-
18:1:ω6c	-	-	13.72	12.48	-	-	22.41	-	-	-	-	-	13.29	-	-
18:1:ω9c	72.38	64.14	41.77	60.28	63.86	69.74	31.56	67.74	42.99	62.28	44.64	70.61	30.28	72.93	49.45
18:1:ω9t alcohol	2.78	-	-	2.17	2.62	-	-	-	2.68	-	-	-	4.24	-	-
18:1:ω12c alcohol	-	-	6.88	-	-	-	10.03	-	-	-	-	-	4.29	-	-
18:0	-	-	-	-	-	1.34	-	1.81	1.92	-	-	-	-	-	-
19:1:ω7c	-	3.79	-	-	2.81	-	-	-	-	-	-	-	-	-	-
20:0	-	-	-	-	-	-	-	-	1.82	-	-	-	-	-	-
21:1:ω7c	-	-	11.86	-	-	-	26.77	-	-	-	-	-	-	-	-
22:0 3 OH	-	-	-	-	-	-	-	-	-	-	-	-	6.91	-	-
24:5:ω3	-	-	-	-	-	-	-	-	2.20	-	-	-	-	-	-
25:0	-	-	-	-	-	-	9.23	-	-	-	-	-	6.33	-	-
26:0 3 OH	7.75	16.18	-	14.23	10.42	-	-	-	5.38	-	25.21	-	-	12.02	-
28:0	-	-	-	-	-	-	-	-	-	-	3.29	-	-	-	-
ISO 17:1 AT9	-	-	5.29	-	-	-	-	-	-	-	-	-	3.95	-	-
ISO 17:1 G	-	-	-	-	2.20	-	-	-	-	-	-	-	-	-	-
C21 primary alcohol	-	-	-	-	-	12.41	1.41	-	1.03	-	-	1.47	-	1.76	-
C25N alcohol	-	-	-	-	-	-	-	-	-	21.67	15.76	12.67	-	-	36.55
Cis9,10epoxy 18:0	-	-	-	-	-	-	-	-	-	4.36	-	-	-	-	6.10

Table 4. Composition of fatty acids of the C population.

Fatty acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
16:0	23.26	13.25	29.19	12.11	19.49	22.57	17.93	21.34	20.94	13.27	26.56	20.67	11.93	21.85	21.87
16:1:ω3c	-	-	-	-	-	-	-	2.57	-	-	2.45	2.13	-	-	-
18:0	-	-	-	-	-	2.39	1.28	-	1.83	-	-	-	-	1.62	1.73
18:1:ω5c	-	-	-	-	-	-	-	-	-	-	-	-	6.54	-	-
18:1:ω9c	76.74	52.46	70.81	58.21	62.79	59.06	45.81	55.32	58.51	46.59	62.31	63.39	71.31	58.75	56.88
18:1:ω9t alcohol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.40
21:1:ω7c	-	13.39	-	-	-	-	-	-	-	-	-	-	-	-	-
19:1:ω7c	-	-	-	-	-	-	2.25	-	-	4.51	-	-	-	-	-
24:0 2 OH	-	-	-	-	-	-	0.96	-	-	-	-	-	-	-	-
25:0	-	4.13	-	-	-	-	-	-	-	-	-	-	-	-	-
26:0 3 OH	-	16.77	-	29.68	17.72	-	-	19.69	6.64	35.64	8.69	13.80	10.22	-	-
28:0	-	-	-	-	-	0.98	-	1.09	-	-	-	-	-	-	-
C21 primary alcohol	-	-	-	-	-	15.00	31.77	-	12.88	-	-	-	-	17.78	13.51

Table 5. Composition of fatty acids of the D population.

Fatty acids	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
16:0	13.25	16.78	-	12.87	-	23.90	18.11	16.76	11.91	13.54	14.37	15.63	27.68	24.55	20.18
16:1:ω3c	-	-	-	-	-	3.51	-	-	-	-	-	-	-	-	-
16:1:ω9	-	-	-	-	-	-	1.76	-	-	-	-	-	-	-	-
18:1:ω9c	66.37	69.79	48.28	57.56	32.01	63.37	65.46	41.96	40.25	-	62.14	44.21	22.95	-	48.86
18:1:ω6c	18.35	-	19.91	-	-	-	-	-	-	3.81	-	9.60	14.62	-	13.25
18:1:ω12c alcohol	-	-	4.79	-	-	-	-	-	-	-	-	-	-	-	-
18:1:ω9t alcohol	-	-	-	-	-	-	3.29	-	-	-	-	-	-	-	-
18:1:ω11c	-	-	-	-	-	-	-	-	-	58.86	-	-	-	-	-
18:3:ω3c alcohol	-	-	4.79	-	-	-	-	-	-	-	-	-	-	-	-
18:3:ω6c	-	-	-	-	-	-	-	8.81	7.27	8.71	-	-	-	-	-
20:0	-	-	-	-	-	-	-	7.12	-	-	-	-	-	-	-
20.3:ω6c	-	-	-	-	30.20	-	-	-	-	-	-	-	-	-	-
19:1:ω7c	-	-	-	-	-	3.14	2.07	-	4.79	-	-	-	-	-	-
21:1:ω7c	-	-	24.38	-	37.80	-	-	-	-	6.33	-	25.81	28.52	30.94	17.71
22:0 3 OH	-	-	-	-	-	-	-	-	-	-	-	4.75	-	30.19	-
25:0	-	-	2.64	-	-	-	-	-	-	-	-	-	6.23	-	-
25:0 3 OH	3.03	-	-	-	-	-	-	-	-	-	-	-	-	14.32	-
26:0 3 OH	-	13.43	-	29.57	-	6.07	9.30	-	30.40	8.74	-	-	-	-	-
28:0	-	-	-	-	-	-	-	2.29	-	-	4.00	-	-	-	-
C20 N alcohol	-	-	-	-	-	-	-	8.33	5.38	-	-	-	-	-	-
C21 primary alcohol	-	-	-	-	-	-	-	14.73	-	-	19.49	-	-	-	-

25:0, and 28:0 are rarely seen and found in low concentrations. 18:1:ω11c had only one individual and a range of 58.86%. Differing from the other population, some individuals were detected to contain 18:1:ω11c, 18:3:ω3c alcohol, 18:1:ω6c, 20:3:ω6c, and C20 N alcohol. Oleic (18:1:ω9c; 22.95 to 69.79% within the population) acid forms the bulk of the total fatty acids found. Biochemical similarity index amongst the individuals of the D population ranged between 0.100 and 0.989. The dendrogram realized from the FAME grouped the 4 populations into two major clusters (Figure 3). Cluster 1 formed populations A and B. Cluster 2 formed populations C and D.

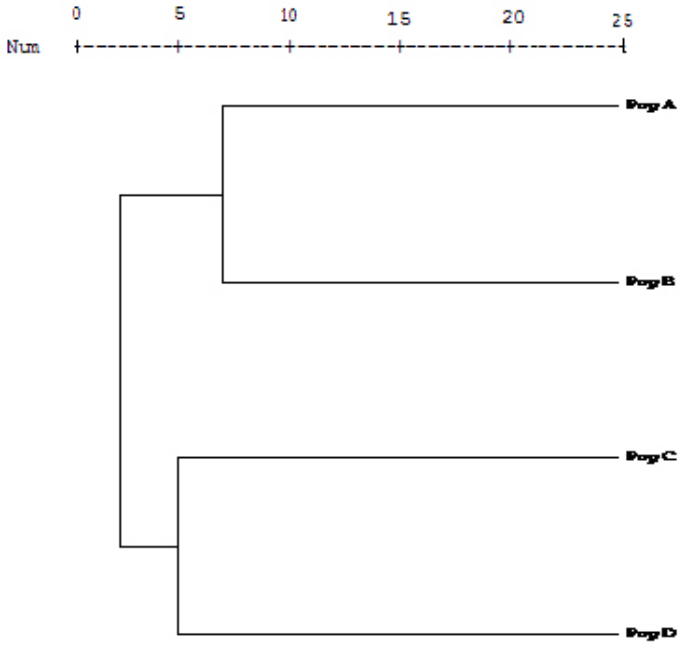

Figure 3. Dendrogram of genetic distances based on FAME 4 populations of *Vitis vinifera* (Kabarcık).

According to the fatty acid profile, 32 different fatty acids were determined and oleic acid had the highest ratio in all the populations studied. The B population contains the highest fatty acids of all the populations. Ecological factors cause the differences observed in fatty acids of plant populations. These factors include temperature, location, soil structure, altitude, rain, average sun radiation, and nutrition (Hou et al., 2006).

According to the dendograms obtained from statiscal analyses of RAPD and fatty acid profiles, the populations, which are close to each other geographically, are also genetically similar. Genetic diversity showed a relationship with the geographical origin. There was an association between biochemical diversity and molecular diversity.

An association between altitudes and genetic diversity of plants has been reported in different studies (Sabrina and Sabri, 1999; George et al., 2001; Erich and Johann, 2002). These studies showed different results owing to the plant materials with different characters (e.g., breeding system, bio-geographical distribution), sampling strategy (on a large or small

geographical scale), and various molecular markers. In macro-scale regions, the reproductive isolation of plants growing at high and low altitude is often observed in favor of high altitude. At the micro-environment level, breeding systems and seed dispersal can influence genetic diversity and differentiation among different populations (Bockelmann et al., 2003).

Clonality and spatial isolation of populations may lower genetic diversity within and increase genetic separation among populations (Barrett and Kohn, 1991; McLellan et al., 1997; Gaudeul et al., 2000; Landergott et al., 2001; Cheon et al., 2002; Despres et al., 2002). Molecular variation within populations was also correlated with the altitude of the population. Changes in population genetic variability due to ecological conditions are rarely observed (Shimizu et al., 2002; Young et al., 2002). The *Parnassia palustris* habitat type affected neither within-population genetic diversity nor genetic and phenotypic differentiation among populations (Bonnin et al., 2002) and in the alpine *Saxifraga oppositifolia* no effect was detected for altitude on genetic population variability (Gugerli et al., 1999). Along environmental gradients, several parameters such as individual age, overlap of generations, or recruitment frequency in climatically favorable years may change and influence intrapopulation genetic variation.

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