AFLP analysis of genetic variation within the two economically important Anatolian grapevine (*Vitis vinifera* L.) varietal groups

Ali Ergül, Kemal Kazan, Sümer Aras, Volkan Çevik, Hasan Çelik, and Gökhan Söylemezoğlu

Abstract: The Anatolian region of modern-day Turkey is believed to have played an important role in the history of grapevine (*Vitis vinifera* L.) domestication and spread. Despite this, the rich grape germplasm of this region is virtually uncharacterized genetically. In this study, the amplified fragment length polymorphisms (AFLP)-based genetic relations of the grapevine accessions belonging to the 2 economically important Anatolian table grape varietal groups known as *V. vinifera* 'Misket' (Muscat) and *V. vinifera* 'Parmak' were studied. Thirteen AFLP primer combinations used in the analyses revealed a total of 1495 (35.5% polymorphic) and 1567 (34.6% polymorphic) DNA fragments for the 'Misket' and 'Parmak' varietal groups, respectively. The unweighted pair-group method with arthimetic averaging (UPGMA) cluster analysis and principal coordinate analysis (PCA) conducted on polymorphic AFLP markers showed that both varietal groups contain a number of synonymous (similar genotypes known by different names) as well as homonymous (genetically different genotypes known by the same name) accessions. Our results also showed that 6 of the Anatolian 'Misket' genotypes were genetically very similar to *V. vinifera* 'Muscat of Alexandria', implying that these genotypes might have played some role in the formation of this universally known grape cultivar. Finally, the close genetic similarities found here between 'Muscat of Alexandria' and *V. vinifera* 'Muscat of Hamburg' support the recent suggestion that 'Muscat of Hamburg' probably originated from 'Muscat of Alexandria' through spontaneous hybridizations. Overall, the results of this study have implications for not only preservation and use of the Anatolian grape germplasm, but also better understanding of the historical role that this region has played during the domestication of grapes.

Key words: 'Misket', 'Parmak', AFLP, *Vitis vinifera* L.

Résumé : L'Anatolie, une région située au sein de la Turquie moderne, est estimée avoir joué un rôle important dans la domestication de la vigne (*Vitis vinifera* L.) et sa propagation. Malgré cela, les abondantes ressources génétiques de cette région n'ont pratiquement pas été caractérisées génétiquement. Dans ce travail, le polymorphisme AFLP (polymorphisme de longueur des produits d'amplification) a été employé pour établir les relations génétiques au sein d'accessions de la vigne appartenant aux 2 groupes de raisin de table qui sont d'importance économique en Anatolie, soit le groupe *V. vinifera* 'Misket' (Muscat) et *V. vinifera* 'Parmak'. Les 13 combinaisons d'amorces AFLP employées au cours de ces analyses ont révélé un total de 1495 (35,5 % polymorphes) et 1567 (34,6 % polymorphes) fragments d'ADN chez les groupes 'Misket' et 'Parmak', respectivement. Une analyse de groupement UPGMA (« unweighted pair-group with arithmetic averaging ») et une analyse des coordonnées principales réalisées sur les marqueurs AFLP polymorphes ont montré que les 2 groupes variétaux contiennent plusieurs synonymes (génotypes semblables connus sous divers noms) de même que des homonymes (des génotypes différents portant le même nom). Ces résultats ont également montré que 6 des gènotypes du groupe 'Misket' étaient très semblables génétiquement au *V. vinifera* 'Muscat of Alexandria', laissant ainsi penser que ces génotypes auraient pu jouer un rôle dans l'évolution de ce cépage connu de tous. Finalement, la grande proximité génétique observée entre le 'Muscat d of Alexandria' et le *V. vinifera* 'Muscat of Hamburg' vient appuyer la récente hypothèse selon laquelle le 'Muscat of Hamburg' serait vraisemblablement dérivé du 'Muscat of Alexandria' suite à des hybridations spontanées. Globalement, les résultats de cette étude ont des

Received 2 June 2005. Accepted 28 November 2005. Published on the NRC Research Press Web site at http://genome.nrc.ca on 6 June 2006.

Corresponding Editor: P. Donini.

A. Ergül. Ankara University, Institute of Biotechnology, 06500 Besevler-Ankara, Turkey.
K. Kazan. Commonwealth Scientific and Industrial Research Organization (CSIRO) Plant Industry, Queensland Bioscience Precinct, St. Lucia, Queensland 4067, Australia.
S. Aras. Ankara University, Faculty of Science, Department of Biology, 06100 Tandogan-Ankara, Turkey.
V. Çevik. Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture, Antakya, Turkey.
H. Çelik and G. Söylemezoğlu.[1] Ankara University, Faculty of Agriculture, Department of Horticulture, 06110 Diskapi, Ankara, Turkey.

[1]Corresponding author (e-mail: soylemez@agri.ankara.edu.tr).

 doi:10.1139/G05-121

implications non seulement pour la conservation et l'utilisation des ressources génétiques d'Anatolie, mais ils contribuent également à comprendre le rôle historique qu'aurait joué cette région dans la domestication de la vigne.

Mots clés : 'Misket', 'Parmak', AFLP, *Vitis vinifera* L.

[Traduit par la Rédaction]

Introduction

The history of the domestication of the cultivated forms of grapes (*Vitis vinifera* L.) has been the subject of many studies. According to one of the widely accepted views, the grape was first domesticated in the Caucasian area, where the highest genetic diversity of wild grape species occurs (Levadoux 1956; Olmo 1995). This view is supported by the fact that the remains of cultivated grape seeds and evidence for wine making were found in Iran as early as the fourth millennium BC (McGovern et al. 1996; McGovern 2003). From this initial region of domestication, grape cultivation is thought to have gradually spread all over the Mediterranean and Europe, possibly by traders and conquerors (e.g., the Romans) (Allaby et al. 1997; Arnold et al. 1998; Bowers et al. 1999). Owing to a unique location between the Caucasus, southwest Asia, and the Mediterranean and Europe, Anatolia, the Asian part of modern-day Turkey, and its surrounding areas probably served as the secondary centers of diversification for current grape varieties. This region has a rich grape germplasm, possibly emerged as a result of natural hybridizations, mutations, and artificial selections over many years (Negrul 1938; Levadoux 1956; McGovern et al. 1995; McGovern 2003; Aradhya et al. 2003). Despite this, to date, very few studies have been conducted on the genetic characterization of Anatolian grape germplasm (Ergül et al. 2002*a*, 2002*b*). It is suspected that some of the older grapevine genotypes of this region currently face extinction. Clearly, better characterization and the preservation of this germplasm would not only contribute to the knowledge on the history of grapevine domestication, but also aid in future breeding studies.

Recently, in an attempt to preserve grape genetic resources, a grape germplasm repository called the National Grapevine Germplasm Vineyard was established at the Institute of Viticulture in Tekirdağ, Turkey. This collection currently contains approximately 1200 grapevine accessions collected from different locations of Anatolia over more than 30 years (Çelik et al. 2005). Initial ampelographic studies conducted on this collection suggested that the Turkish grapevine varieties locally known as the 'Amasya', 'Dimrit', 'Gemre', 'Misket', 'Parmak', and 'Razakı' groups, may all potentially contain a large number of homonyms (genetically different genotypes maintained under the same name) and synonyms (genetically identical genotypes known by different names). The presence of such genotypes, which are commonly encountered in almost all grape growing regions of the world, prevents standardization at the multiplication stage and causes greatly redundant germplasm in the collection. However, genetic studies based on a limited number of ampelographic characteristics do not always allow reliable discrimination of such genotypes. By contrast, DNA markers, which show independence from the developmental stage, and environmental factors provide highly discriminatory information and, therefore, are commonly used for cultivar and clone identification and parentage analyses. Randomly amplified polymorphic DNAs (RAPDs) (This et al. 1997; Ye et al. 1998; Vidal et al. 1999), amplified fragment length polymorphisms (AFLPs) (Cervera et al. 1998; Scott et al. 2000; Fossati et al. 2001; Fanizza et al. 2003), and simple sequence repeats (SSRs) (Thomas and Scott 1993; Bowers et al. 1996; Sefc et al. 1999; Lefort and Roubelakis-Angelakis 2001; Vouillamoz et al. 2003; Aradhya et al. 2003) have been used previously in *V. vinifera*. However, to best of our knowledge, AFLP markers have not been used previously for the characterization of Anatolian grape germplasm, particularly for the identification of homonyms and synonyms therein.

As a part of our ongoing efforts to genetically characterize the Anatolian grape germplasm, this AFLP study was undertaken. The aim was to determine the genetic relatedness of selected accessions within the economically important 'Misket' and 'Parmak' varietal groups. Of these, the 'Misket' group grapes are of particular importance as table grapes owing to their distinct muscat flavor, whereas the 'Parmak' group grapes are preferred by the growers owing to their high yield capacity, especially when grown in unirrigated areas. Grapevine accessions belonging to these 2 varietal groups constitute approximately 5% of the total grape production in Turkey, which, with an average production of 3.6 million tonnes/year, is the 4th largest grape producer in the world (Çelik et al. 2005). The word *Misket* used to describe Muscat-group grapes means "marble or round" in Turkish and is probably derived from the word "Muscat". The Turkish word *Parmak* means "finger" in English and is indicative of the long, ellipsoidal berry shape of the 'Parmak'-group grape accessions. Our results reported here showed that although none of the grape accessions studied within each varietal group was genetically identical, a number of accessions with different names were very closely related, suggesting that these accessions are probably synonymous. In addition, we identified accessions, which despite being genetically relatively divergent, were known by the same names, suggesting that these are possibly homonymous. These results have implications in better management and use of Anatolian grape germplasm resources.

Materials and methods

Plant material

The leaves and shoots of the grape accessions studied in this paper were obtained from the National Grapevine Germplasm Vineyard at the Institute of Viticulture in Tekirdağ, Turkey. Some of the important characteristics of these accessions are presented in Table 1.

Table 1. Some ampelographic characteristics of the 'Parmak' and 'Misket' group grape accessions used in this study.

Accession No.	Genotype name	Location (town/city/region)	Cluster form	Berry form	Berry color	Flavor	Seed no.
Misket accessions							
M1–243.22	'Beyaz Misket'	Havsa /Edirne/Thrace	Conical	Oblate	White	Neutral	2
M2–305.59	'Misket'	Şarköy/Tekirdağ/Thrace	Cylindrical	Round	White	Muscat	2–3
M3–447.45	'Beyaz Misket'	Gördes/Manisa/Aegean	Shouldered conical	Falcoid	White	Neutral	1–2
M4–488.45	'Siyah Misket'	Gördes/Manisa/Aegean	Winged conical	Round	Black	Muscat	2
M5–489.45	'Siyah Misket'	Kırkağaç/Manisa/Aegean	Cylindrical	Round	Black	Muscat	3
M6–493.45	'Beyaz Misket'	Gördes/Manisa/Aegean	Conical	Ellipsoidal	White	Muscat	3–4
M7–541.20	'Misket'	Acıpayam/Denizli/Aegean	Conical	Round	White	Neutral	1–2
M8–649.48	'Kokulu Uzüm'	Ula/Muğla/Aegean	Winged conical	Obovoid	White	Neutral	1–2
M9–724.22	'Yatık Beyaz'	Center/Edirne/Thrace	Conical	Round	White	Sweet	2
M10–303.59	'Misket'	Şarköy/Tekirdağ/Thrace	Conical	Round	White	Sweet	3
M11	'Muscat of Alexandria'	University of Ankara, Faculty of Agriculture, Viticulture Research Station	Shouldered-winged conical	Obovoid	White	Muscat	2–3
M12	'Muscat of Hamburg'	University of Ankara, Faculty of Agriculture, Viticulture Research Station	Winged conical	Ovate	Purplish black	Muscat	2–3
Parmak accessions							
P1-79.01	'Hatun Parmağı'	Saimbeyli/Adana/Mediterranean	Conical	Olivoid	Whitish Pink	Sweet	3
P2-118.31	'Hatun Parmağı'	Hassa/Antakya/Mediterranean	Winged conical	Ellipsoidal	Black	Sweet	2–3
P3-298.59	'Kadın Parmagı'	Şarköy/Tekirdağ/Thrace		Ellipsoidal	Pink	Neutral	1–2
P4-330.37	'Parmak'	Tosya/Kastamonu/Western Blacksea	Shouldered conical	Long ovoid	Black	Sweet	2
P5-423.42	'Kadın Parmağı'	Hadım/Konya/Central Anatolia	Conical	Ellipsoidal	Pink	Sweet	3
P6-427.42	'Kadın Parmağı'	Merkez/Konya/Central Anatolia	Shouldered conical	Long ellipsoidal	Black	Sweet	2
P7-464.42	'Kızıl Parmak'	Bozkır/Konya/Central Anatolia	Cylindrical	Round	Red	Sweet	
P8-467.42	'Kızıl Parmak'	Bozkır/Konya/Central Anatolia	Conical	Round	Whitish Pink	Neutral	3
P9-611.06	'Parmak'	Kalecik/Ankara/Central Anatolia	Conical	Long ellipsoidal	White	Neutral	1-2
P10-639.48	'Kadın Parmağı'	Ula/Muğla/Aegean	Conical	Long falcoid	Whitish Pink	Sweet	1
P11-642.48	'Kadın Parmağı'	Fethiye/Muğla/Aegean	Conical	ellipsoidal	White	Neutral	1
P12-721.67	'Kara Parmak'	Safranbolu/Zonguldak/Western Blacksea	Shouldered conical	Long round	Whitish Pink	Sweet	2-3
P13-750.67	'Parmak'	Safranbolu/Zonguldak/Western Blacksea	Winged conical	Long ellipsoidal	White	Neutral	2
P14-754.67	'Akparmak'	Safranbolu/Zonguldak/Western Blacksea	Cylindrical	Round	White	Sweet	1
P15-908.27	Hatun Parmağı'	İslahiye/Gaziantep/Southeast Anatolia	Winged Cylindrical	Round	White	Neutral	3-4

Table 2. The numbers of total and polymorphic DNA fragments generated by the selected primer combinations used in the AFLP analysis of the 'Misket' and 'Parmak' groups of Anatolian grape *(Vitis vinifera* L.) accessions.

Primer combination		'Misket' group			'Parmak' group		
*Eco*RI+2-	*Mse*I+3-	Total bands	Polymorphic bands	% polymorphism	Total bands	Polymorphic bands	% polymorphism
AT	CAC	95	23	24.2	120	40	33.3
	CTC	133	50	37.6	115	31	27.0
	CTG	93	25	26.9	125	40	32.0
TG	CAC	132	65	49.2	108	40	37.0
	CTC	98	41	41.8	100	43	43.0
AC	CAT	110	41	37.3	145	42	29.0
	CAC	130	43	33.1	112	42	37.5
TC	CAC	112	32	28.6	139	53	38.1
	CTC	130	43	33.1	141	60	42.6
	CTG	109	33	30.3	115	38	33.0
TT	CAC	117	48	41.0	124	39	31.5
	CTA	110	40	36.4	113	38	33.6
	CTC	126	46	36.5	100	36	36.0
Total and (or) average		1495	530	35.5	1567	542	34.6

DNA isolation

DNA was extracted from fresh, young leaf tissue with the isolation protocol by Lodhi et al. (1994). Concentration and purity of the DNA extracted were determined by electrophoresis on 1% *w*/*v* agarose gels, and spectrometrically at 260 nm. Total DNA was diluted to a working concentration of 100 ng/μL.

AFLP analysis

AFLP analysis was performed essentially as described by Vos et al. (1995) using the adapter and PCR primer sequences and PCR cycles described therein. Total genomic DNA (0.25 μg) was digested with 2.5 U *Eco*RI and 2.5 U *Mse*I in 25 μL reaction mixtures containing 10 mmol/L Tris–HCl (pH 7.5), 10 mmol/L magnesium acetate, and 50 mmol potassium acetate for 4 h at 37 °C. Following digestion, the mixture was incubated at 70 °C for 15 min to inactivate the restriction endonucleases. Then, 25 μL of a mixture containing 5 pmol *Eco*RI adapter (5′-CTCGTAGACTGCGTACC and CTGACGCATGGTTAA-5′), 50 pmol *Mse*I adapter (5′-GACGATGAGTCCTGAG and TACTCAGGACTCAT-5′), and 1U T4 DNA ligase were added and the ligation reaction was incubated at 15 °C overnight. The pre-amplification reaction was performed with 5 μL of template DNA (1:10 solution diluted from the ligation mixture with $T_{10}E_{0.1}$ (10 mmol/L Tris–HCl (pH 8.0) and 0.1 mmol/L EDTA) using a pair of primers based on the sequences of the *Eco*RI and *Mse*I adapters, including 1 additional selective nucleotide at the 3′ end of the *Mse*I primer (*Mse*I+C: 5′-GATGAGTCCTGAGTAA/C-3′) and the *Eco*RI primer (*Eco*RI+A: 5′-GACTGCGTACCAATTC/A-3′). Following pre-amplification, PCR products were diluted 50 fold with $T_{10}E_{0.1}$. Five microlitres of this solution was used as template for a second round of 20 μL PCR, primed with *Eco*RI+2 and *Mse*I+C+2 oligomers (Table 2). The *Eco*RI+2 primers were labelled by phosphorylating the 5′ end with [γ^{33}P]ATP for fragment detection (Vos et al. 1995). The PCR products were mixed with an equal volume of tracking dye (98% formamide, 10 mmol/L EDTA, 0.05% bromphenol blue, and xylene cyanol), denatured at 94 °C for 3 min, and immediately cooled on ice. Aliquots (5 μL) of each reaction were loaded onto denaturing polyacrylamide gels. Gels were dried and exposed overnight to Kodak Biomax MR-2 film at room temperature.

Data analysis

Polymorphic AFLP bands were scored manually as present (1) or absent (0) across all 'Parmak' and 'Misket' group genotypes for each primer-pair combination and the values were used to compile a binary data matrix. The MVSP software package version 3.1 (Kovach 1999) was used to calculate Jaccard's (Jaccard 1908) similarity coefficients among the genotypes as follows:

$$S_{ij} = \frac{N_{ij}}{N_{ii} + N_{ij} + N_{jj}}$$

where S_{ij} is the similarity index between *i*th and *j*th genotype; N_{ij} is the number of bands present in both genotypes, N_{ii} is the number of bands present in *i*th genotype, but absent in *j*th genotype; and N_{jj} is the number of bands present in *j*th genotype, but absent in *i*th genotype. A dendogram was constructed using the unweighted pair-group method with arithmetic averaging (UPGMA). Robustness of nodes was estimated by bootstrap analysis of 1000 permuted datasets that were generated using WinBoot software (Yap and Nelson 1996). PCA was also carried out to show multiple dimensions of the distribution of the genotypes in a scatter plot.

Results

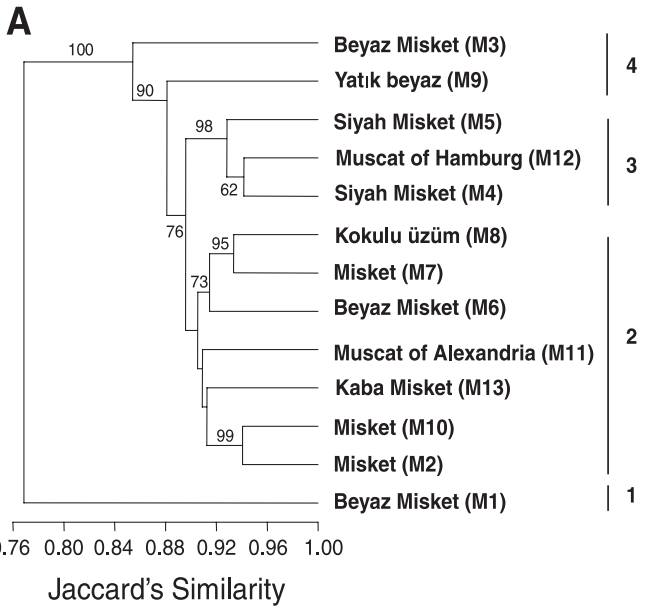
In this study, AFLP markers were used in the analysis of genetic relationships of 13 'Misket' and 15 'Parmak' accessions. Within the 'Misket' group accessions examined, the accessions M1, M3, and M6 are known as 'Beyaz Misket' ('White Muscat'); M4 and M5 as 'Siyah Misket'('Black Muscat'); and M2, M7, and M10 as 'Misket' (Table 1). The

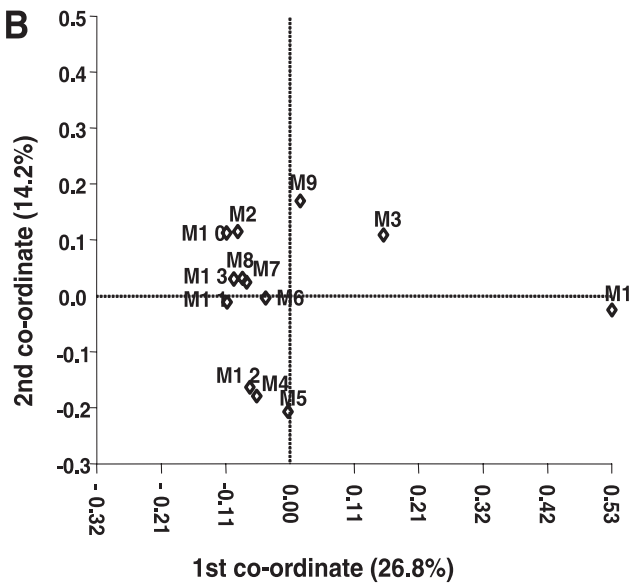
remaining accessions (M9, M8, and M13) were individual 'Misket'accessions known by other names. Also included in our analysis are 2 well-known Muscat cultivars: 'Muscat of Alexandria' (M11) and 'Muscat of Hamburg' (M12). Similarly, several 'Parmak' accessions (e.g., 'Hatun Parmağı', 'Kadın Parmağı', 'Parmak', and 'Kızıl Parmak'), which are all known by either the same or similar names (e.g., *hatun* = *kadın* = woman) despite their ampelographic differences (Table 1), were selected for AFLP analysis.

We first assessed the level of overall genetic similarities between 'Parmak' and 'Misket' groups to confirm that accessions belonging to these 2 groups classified previously based on their ampelographic characteristics were indeed genetically different. For this analysis, we used 3 'Misket' (i.e., M9, M10, and M11) and 4 'Parmak' (P3, P8, P11, and P12) accessions and estimated their genetic relatedness using 3 AFLP primer pairs (i.e., *Eco*RI-TC–*Mse*I-CAC, *Eco*RI-TG–*Mse*I-CTC and *Eco*RI-TC–*Mse*I-CTC). In the dendogram generated based on Jaccard's genetic similarities (Jaccard 1908), these 3 'Misket'accessions, including 'Muscat of Alexandria', formed a distinct cluster, whereas 4 'Parmak' accessions clustered together (data not shown). As expected, the overall genetic similarity between 'Misket' and 'Parmak' groups of accessions was low (0.260) (data not shown).

To assess genetic variation among the accessions within each varietal group, we used 13 AFLP primer pairs that produced a total of 1495 DNA bands with 35.5% polymorphism in the 'Misket' group and a total of 1567 DNA bands with 34.6% polymorphism in the 'Parmak' group. Subsequently, a total of 530 and 542 unambiguous polymorphic DNA bands varying between 50 and 500 bp in size were used, respectively, for the genetic analysis of 'Misket' and 'Parmak' group accessions. The number of polymorphic bands generated per primer pair varied between 23 and 65 for 'Misket' and between 31 and 60 for 'Parmak' group accessions (Table 2). The Jaccard genetic similarities between pairs of genotypes in each group were then used to generate a dendogram based on the UPGMA analysis for both groups of accessions (Figs. 1A and 2A). Pair-wise similarities between the accessions ranged from 0.755 to 0.942 in the 'Misket' group. The lowest genetic similarity (0.755) was found between M1 and M2-M10, whereas the highest genetic similarity (0.942) was obtained between M4-M5 and M4-M12. This was followed by the M2-M10 and M7-M8 groups with genetic similarities of 0.941 and 0.934, respectively. The dendrogram generated identified 4 major subclusters containing 1, 7, 3, and 2 accessions for the 'Misket' group (Fig. 1A). Sub-cluster 1 contained only the accession M1 ('Beyaz Misket'), which was distantly related to the rest of the accessions. Sub-cluster 2 contained 7 'Misket' accessions. Two 'Siyah Misket' accessions (M4-M5) and 'Muscat of Hamburg' (M12), all having a black berry color. A number of other 'Misket' accessions (M8, M7, M6, M13, M10, and M2) including 'Muscat of Alexandria' (M11), having a white berry color, formed separate groups within subclusters 2 and 3 (Fig. 1). Finally, accessions M3 and M9 formed sub-cluster 4. The genetic similarity between the 2 relatively well-known 'Muscat' cultivars, namely, 'Muscat of Alexandria' (M11) and 'Muscat of Hamburg' (M12), was 0.915. Overall, accessions that were originated from close geographical locations were genetically more similar than

Fig. 1. (A) UPGMA cluster analysis of 'Misket' group accessions. Bootstrap *p* values above 50% are indicated at the corresponding node for each cluster. (B) Principal co-ordinate analysis (PCA) of 'Misket'group accessions.

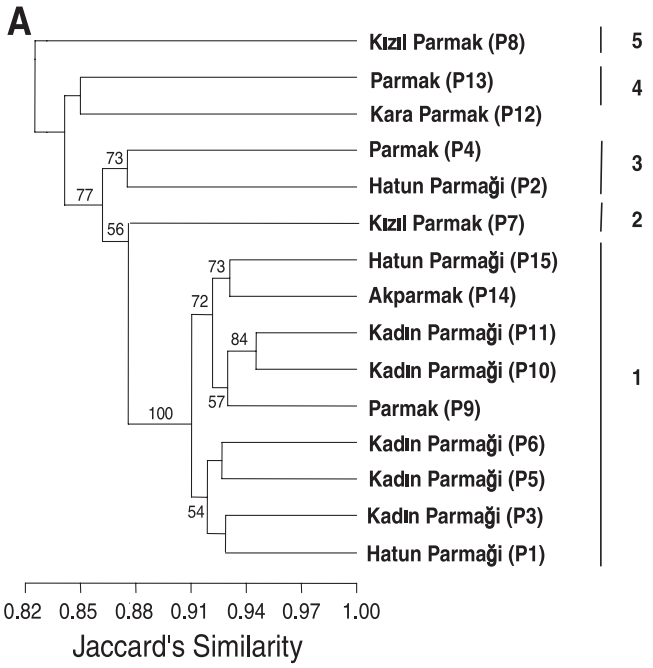


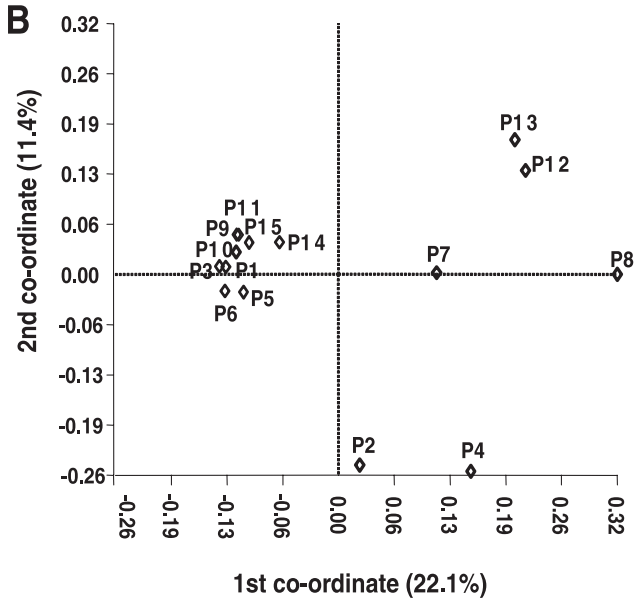


those of relatively diverse locations for all 'Misket' accessions examined (Table 1; Fig. 1).

The genetic diversity measured by AFLP analysis among the 'Parmak' group accessions appeared to be greater than that among the 'Misket' group accessions. The lowest genetic similarity (0.808) was between 'Kızıl Parmak' (P8) and the sub-cluster containing 4 'Kadın Parmağı' accessions (P6, P5, P3, and P1); the highest genetic similarity (0.945) was observed between 2 'Kadın Parmağı' accessions (P10 and P11) of sub-cluster 1. In the dendogram shown in Fig. 2A, 9 'Parmak' accessions (P15, P14, P11, P10, P9, P6, P5, P3, and P1) were genetically more related and formed a single group. P4 and P2 with a pair-wise genetic similarity of 0.875

Fig. 2. (A) UPGMA cluster analysis of 'Parmak' group accessions. Bootstrap *p* values above 50% are indicated at the corresponding node for each cluster. (B) Principal co-ordinate analysis (PCA) of 'Parmak' group accessions.





and P13 and P12 with a pair-wise genetic similarity of 0.850 formed sub-clusters 3 and 4, respectively.

PCA yielded scatter plots that are similar to those produced by cluster analysis for both groups (Figs. 1B and 2B). The principal co-ordinates 1, 2, and 3 explained 52% of the total variation with each co-ordinate contributing 26.8%, 14.2%, and 11.5%, respectively, of the variation in the 'Misket' group. Two main groups and subgroups observed in the dendogram appeared to be well separated. 'Muscat of Alexandria' (M11) and 6 accessions with white berry color clustered together in the same group were located together in the upper-left quadrant. 'Muscat of Hamburg' (M12), M4, and M5 were grouped in the lower-left quadrant, whereas M9 and M3 were grouped in the upper-right quadrant. M1 was distinct from the rest of the genotypes (Fig. 1B). For the 'Parmak' group, up to 43% of the total variation was explained by the first 3 axes, which accounted for 22.1%, 11.4%, and 9.3% of the variation, respectively. Accessions P15, P14, P11, P10, P9, P6, P5, P3, and P1 were located in the upper- and lower-left quadrants. Other genotypes showed a distribution in the upper- and lower-right quadrants, which is consistent with their groupings in the dendogram (Fig. 2B).

Discussion

Identification of genetically related genotypes and (or) accessions within a grape variety based on a limited number of morphological and ampeolographic characteristics has proven difficult in this clonally propagated plant species. Therefore, molecular markers have been widely employed in germplasm characterization and preservation in *Vitis* (Ye et al. 1998; Vidal et al. 1999; Cervera et al. 1998; Fanizza et al. 2003; Bowers et al. 1996; Sefc et al. 1999; Aradhya et al. 2003). In fact, application of AFLPs and SSRs in determining homonyms and synonyms within the grapevine varietal group 'Schiave' has been previously reported (Fossati et al. 2001). The results presented here also show the usefulness of AFLP markers in detecting intravarietal genetic variation within the 2 economically important table-grape groups. To the best of our knowledge, this is the first report aimed at detailed characterization of the 'Misket' and 'Parmak' varietal groups of Anatolia, a region with a rich history of grapevine cultivation.

The 'Misket' and 'Parmak' group grape accessions studied here are known to be genetically different from one another based on their morphological and ampeolographic characteristics (Table 1). Our results from the AFLP analyses support this conclusion, as we found lower genetic similarity between these varietal groups than within the groups. Interestingly, however, our results also clearly showed that none of the grape accessions examined within each varietal group was completely identical. For instance, a relatively small amount of genetic variation was still detectable between the 2 'Misket' accessions M2 and M10, although these 2 accessions were originated from the same region and also known by the same name. The ampelographic characteristics of M2 and M10 also showed some differences. For instance, as shown in Table 1, in contrast to M2, which has a distinct Muscat flavor, M10 has a sweet flavor. This suggests that morphological and ampeolographic characteristics can be of additional value in characterizing this germplasm when used in conjunction with the molecular data.

However, one of the difficulties encountered in reliable detection of synonym grape genotypes lies in determining the minimum level of genetic similarity above which 2 genotypes are presumed to be identical. Cervera et al. (1998) proposed that grape genotypes with a genetic similarity of 90% or above should be considered cultivars of the same variety, provided that sufficient numbers of AFLP loci are analyzed. Ulanovsky et al. (2002) have also used a similar genetic sim-

ilarity value in detection of synonyms in grapevines. Consistent with these previous studies, we considered 2 accessions as synonyms when the genetic similarity between these accessions was 0.900 or higher. In addition, our results suggest that the reliable discrimination of genetically related genotypes within each varietal group was dependent on the use of a relatively high number of AFLP primers. Previously, different researchers had employed varying numbers of AFLP primers to examine the genetic relationships among grape accessions. For example, Cervera et al. (1998) stated that even a single primer pair would be sufficient for discrimination of grape genotypes at the varietal level, whereas 2 or more primer pairs may be required for reliable discrimination of clonal entities. Fossati et al. (2001) and Labra et al. (2001) used 4 AFLP primer pairs in their analyses of the 'Schiave' and 'Trebbiano' groups of Italian grape genotypes. Imazio et al. (2002) used 3 primer combinations in the analyses of 'Traminer' clones, whereas Fanizza et al. (2003) used 22 primer combinations in the analyses of aromatic grapevines. In this study, when we compared the dendograms generated using either 4 or 13 primer pairs, we observed slightly different clustering patterns in the subclusters containing highly related accessions (data not shown). This suggested that additional primers would be needed for better discrimination of accessions with relatively high genetic similarities.

Within the 'Misket' group, accessions M1, M3, and M6 were clearly and sufficiently different genetically despite the fact that these accessions are all known by the same name, 'Beyaz Misket', which is indicative of their white berry color. Thus, these accessions should be regarded as homonyms. In contrast, accessions 'Beyaz Misket', 'Misket', and 'Kokulu Üzüm' (M6, M7, and M8) were all genetically very similar (Figs. 1A and 1B), with genetic similarity values of 0.900 or higher, despite being known by different names; this suggests that these accessions are probably synonymous. Our results also clearly showed that accessions M2 and M10 ('Misket'), as well as M4 and M5 ('Siyah Misket'), are genetically very similar and thus are probably clones derived from the same or related individuals. The close geographical origins and similar ampelographic characteristics of these accessions are also consistent with this conclusion. Interestingly, relatively high genetic similarities observed between M4 and M12 (0.942) and M5 and M12 (0.915) also suggest that 'Siyah Misket' accessions M4 and M5 are likely to be synonyms of 'Muscat of Hamburg' (M12). This conclusion is again supported by the morphological characteristics shared by all 3 accessions. Similarly, 6 'Misket' accessions within sub-cluster 1 showed significant genetic similarity (0.900 or higher) to 'Muscat of Alexandria' (M11). Based on these results, it is tempting to speculate that these accessions and 'Muscat of Alexandria' may have derived from common or related grapevine genotypes. The geographical location of Anatolia between the Transcaucasian region, where *V. vinifera* was first domesticated, and the Mediterranean, and the historical role that this region has played in the spread of grape germplasm, supports this hypothesis. Alternatively these Anatolian 'Misket' genotypes may be earlier forms of grape genotypes that have given rise to 'Muscat of Alexandria' by spontaneous hybridizations. Spontaneous hybridizations are suggested to have played a significant role in the emergence of successful grapevines. For instance, the famous grapevine 'Cabernet Sauvignon' was found to be the progeny of 2 other Bordeaux cultivars, 'Cabernet Franc' and 'Sauvignon Blanc' (Bowers and Meredith 1997). Additional sampling and molecular analysis of grape genotypes from these regions may be required to determine the possible relationships between Anatolian 'Misket' genotypes and 'Muscat of Alexandria' with some certainty. Finally, our results also revealed a genetic similarity value of 0.915 between 'Muscat of Hamburg' (M12) and 'Muscat of Alexandria' (M11). Previously, Stavrakakis and Biniari (1998), using RAPD analysis, reported a similar genetic similarity value (0.864) between these 2 grape cultivars. In addition, Crespan and Milani (2001), and Crespan (2003) have recently suggested that 'Muscat of Alexandria' is the male parent of 'Muscat of Hamburg' and our results are consistent with this suggestion.

The morphological and AFLP data also indicated the presence of synonymous accessions within the 'Parmak' group accessions. For example, the pair-wise genetic similarities among 9 accessions grouped together in sub-cluster 1 were at least 0.900 or higher. Although 2 'Kadın Parmağı' accessions (P5 and P6) originating from the same region showed very high genetic similarity (0.927) and were considered synonyms, they had different berry colors, which might have arisen through mutations affecting only one (e.g., berry color) or a few traits. Six other 'Parmak' accessions (P7, P2, P4, P12, P13, and P8) with genetic similarities of less than 0.900 formed separate clusters. Among these, P4 ('Parmak') and P3 ('Kadın Parmağı'), as well as 2 'Kızıl Parmak' accessions (P7 and P8), are clearly homonyms.

In conclusion, our AFLP analysis of the Anatolian 'Misket' and 'Parmak' grapevine genotypes showed the presence of intra-varietal genetic variation between the 2 groups and allowed potential homonyms and synonyms to be identified. This latter knowledge will be particularly useful in germplasm preservation and management. The relatively distant genotypes identified here within each varietal group can be used in breeding programs to take advantage of heterosis, which is proposed to have played a significant role in the emergence of successful grape cultivars (Bowers et al. 1999). Future analysis of the grapevine genotypes characterized here by means of the standard set of microsatellite reference alleles recently developed by This et al. (2004) would be useful for potential comparisons with previously described grapevine genotypes. Finally, the genetic characterization and comparative analysis of other Anatolian grapevine varieties along with those of previously characterized genotypes from the surrounding regions would shed additional light into the history of domestication of this important plant species.

Acknowledgements

This study is partially funded by the State Planning Organization of Turkey (Project No. 2002-K-120130-11) and Ankara University, Institute of Biotechnology (Project Nos. 14 and 68). We thank Y. Boz and C. Özer, who provided 'Misket' and 'Parmak' accessions used in this study.

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