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Characterization of Autochthonous Grapevine Cultivars (*Vitis vinifera* L.) from the Aegean Region of Turkey Using Simple Sequence Repeats (SSRs)

Burçak İŞÇİ[a], Yıldız DİLLİ[b]

[a]*Ege University, Faculty of Agriculture, Department of Horticulture, 35100, Izmir, TURKEY*
[b]*Manisa Viticulture Research Station, 45040, Manisa, TURKEY*

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Corresponding Author: Burçak İŞÇİ, E-mail: burcak.isci@ege.edu.tr, Tel: +90 (232) 311 26 32
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ABSTRACT

Thirty-six autochthonous grapevine cultivars from the Aegean region of Turkey in addition to standard cultivars Cabernet Sauvignon and Merlot (*Vitis vinifera* L.) were fingerprinted using SSR markers to assess their genetic relationships. Eleven SSR primers produced successful amplifications and yielded 37 polymorphic bands. The number of bands per primer changed between 2 and 6 while the number of polymorphic bands was between 2 and 3.6. Dice genetic similarity coefficients ranged between 0.296 and 0.882 among the genotypes. The UPGMA dendrogram showed two main groups. The first group was composed of Sultan Dimriti, Veyis and Güvercin Gözü cultivars. A large number of sub-groups were placed in the second group which included the majority of autochthonous cultivars. The genetic differences among the autochthonous cultivars and reference cultivars were clearly observed. The results showed that cultivars Siyah Razakı and Parmak (0.96) were the most similar ones. Synonyms were identified for İnek Memesi and Ufak Dimrit cultivars. In addition, homonymous cultivars were detected. SSR markers have proved to be an efficient tool for fingerprinting grapevine cultivars and conducting genetic diversity studies.

Keywords: *Vitis vinifera* L.; Autochthonous grapevine cultivars; Molecular marker; Simple sequence repeat (SSRs)

Ege Bölgesi Yerel Asma Çeşitlerinin (*Vitis vinifera* L.) Basit Tekrar Dizileri (SSRs) ile Karakterizasyonu

ESER BİLGİSİ
Araştırma Makalesi
Sorumlu Yazar: Burçak İŞÇİ, E-posta: burcak.isci@ege.edu.tr, Tel: Tel: +90 (232) 311 26 32
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ÖZET

Türkiye'nin Ege bölgesinde yetiştirilen otuz altı yerel asma çeşidi ile standart çeşitlerden Cabernet Sauvignon ve Merlot arasındaki genetik ilişkiler SSR markörleri kullanılarak belirlenmiştir. Kullanılan 11 SSR primeri başarılı amplifikasyonlar vermiş ve toplam 37 polimorfik bant üretmiştir. Bant sayısı primer başına 2 ile 6 arasında ve polimorfik bant sayısı 2

ile 3.6 arasında değişmiştir. Genotipler arasındaki Dice genetik benzerlik katsayıları 0.296 ile 0.882 arasında değişim göstermiştir. UPGMA dendrogramında iki ana grup ortaya çıkmıştır. Birinci grup Sultan Dimriti, Veyis ve Güvercin Gözü çeşitlerinden oluşmuştur. Yerel çeşitlerin büyük kısmının yer aldığı ikinci grup çok sayıda alt gruptan oluşmuştur. Yerel asma çeşitleri ve referans çeşitler arasındaki genetik farklılıklar açıkça gözlenmiştir. En yüksek (0.96) genetik benzerliğin Siyah Razakı ve Parmak çeşitleri arasında olduğu belirlenmiştir. İnek Memesi ve Ufak Dimrit çeşitlerinin birbirinin sinonimi olduğu tespit edilmiştir. Ayrıca homonim çeşitlerin varlığı da ortaya çıkarılmıştır. Sonuçlar, SSR markörlerinin asma çeşitlerinin parmak izi ve genetik çeşitlilik çalışmalarında etkin bir araç olduğunu göstermektedir.

Anahtar Kelimeler: *Vitis vinifera* L.; Yerel üzüm çeşitleri; Moleküler markör; Basit tekrar dizileri (SSRs)

1. Introduction

Turkey is geographically located in the gene centre for grapevines and it is the place of its initial cultivation, viticulture and winemaking undertaken since 6000−5000 BC (Alleweldt & Possingham 1988). The country is composed of several regions and each region possesses local cultivars which are different in color, taste, shape etc. Synonymous are also widely used in these regions. Correct identification of these cultivars is of great importance in cultivar standardization and determination of total cultivar numbers (Ergül et al 2006; Hizarci et al 2012).

The Aegean region plays an important role in Turkish viticulture. Ecological conditions and geographical location of the region creates excellent environment for viticulture. Its production constitutes 33% of the total vineyard area and 43% of the country's grape production. The region is rich in grape germplasm that a number of autochthonous cultivars originated in the area which should be incorporated into grape-breeding programs. Current plant genetic sources are decreasing as a result of environmental and other pressures. Identification of grapevine varieties using molecular techniques and assessment of the accurate naming is crucial for breeding purposes, the international exchange of genetic resources and in grapevine saplings certification systems (Dilli 2008). Several studies have been done on genetic variation within grapevines using different molecular marker systems. Genetic marker analysis is used for the selection of plants in plant breeding programs with desired characteristics (Brown & Kresovich 1996; Özcan et al 2001). Simple sequence repeat (SSR) markers have been widely used to determine the genetic diversity within grapevine cultivars (De Mattia et al 2009; Leao et al 2009). SSR markers have also been used for the correct identification of synonyms and homonyms in breeding studies (Thomas & Scott 1993; Bowers et al 1996; Scott et al 2000; Costantini et al 2005). Taken together with their co-dominant nature and reproducibility, SSR's are associated with specific genetic regions which enable the correct identification of grape cultivars and an assessment of the accuracy of plant names, which are crucial in breeding studies, determining international genetic resource changes and grapevine saplings certification systems (Regner & Wiedeck 2006). SSR primers of core set, VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79 have been recommended for the direct comparison of results from different laboratories (This et al 2004). The International Grape Community has focused on the development of SSR markers and the construction of several genetic maps covering most of the grapevine genome (Vezzulli et al 2008). It is important to identify the genetic potential of local grapevines in order to conserve them within national and international contexts and to incorporate into breeding programs. This will help to improve viticulture and to increase its contribution to the Turkish national economy

In this study, genetic relationships among autochthonous grape cultivars from the Aegean region and two standard cultivars were assessed by using SSR markers. The results would be useful for the selection and more efficient utilization of local germplasm within grape breeding programs.

2. Material and Methods

2.1. Plant material

Leaf samples of the 36 autochthonous grapevine cultivars plus two reference cultivars Cabernet Sauvignon and Merlot (*Vitis vinifera* L.) as reference were either collected from different parts of the Aegean region or supplied by Manisa Viticulture and Research Station. Some important ampelographic traits of these cultivars described based on International Union for the Protection of New Varieties of Plants (UPOV) guidelines are given in Table 1.

2.2. DNA extraction

High quality genomic DNA was extracted from young leaf tissues using the CTAB method modified by Lodhi et al (1994). RNAse treatment was performed on the eluted DNA samples. Concentration and purity of the DNA were determined by NanoDrop® ND-1000 spectrophotometer readings. The DNA was diluted to a working concentration of 10 ng μL^{-1}.

2.3. SSR analysis

Eleven SSR markers employed in the "*European Project GENRES #08"*, and "*2nd Edition of the OIV Descriptor list for grape varieties and Vitis species"* (VIVC 2007; GENRES 2014; OIV 2014) were used in Polymerase Chain Reaction (PCR) amplifications. These primers were VVS3, VVS1, VVS4 and VVS2 (Thomas & Scott 1993), VVMD5, VVMD6 and VVMD7 (Bowers et al 1996), VVMD17 and VVMD28 (Bowers et al 1999), and VrZAG29 and VrZAG79 (Sefc et al 1999). PCR was performed in a final volume of 25 µL containing 5-10 ng of DNA template, 1x PCR reaction buffer (*Fermentas, Life Sciences*), 1.5 mM $MgCl_2$ (*Fermentas, Life Sciences*), 0.2 mM for each dNTP, 0.5 µM each primer, 0.25 Unit Taq DNA polymerase (*Fermentas, Life Sciences*) and milliQ water. The reactions without DNA were included as negative controls. Amplification was performed using PTC-100TM (*MJ Research Inc*.) thermocycler with 2 min initial denaturation/ activation step, followed by 40 cycles at 92 °C for 30 s, 52–56 °C for 1 min and 72 °C for 2 min with a final extension step of 7 min at 72 °C. A DNA ladder (100 bp) was used for the approximate quantification of the bands. Amplified DNA fragments were separated on 6% (w/v) polyacrylamide gel (*Promega Silver Staining kit*) which were silver stained to observe the bands then, the images were recorded by scanning.

2.4. Genetic analysis

The bands (alleles) were scored as present (1) or absent (0) across all the genotypes. Estimates of genetic similarity between pairs were calculated using Dice coefficient (Sneath & Sokal 1973) which were used for SAHN cluster analysis using unweighted pair group method of arithmetic averages (UPGMA) and a dendrogram was generated using the NTSYS (version 2.0) statistical package program (Rohlf 1998).

3. Results and Discussion

SSR analysis was performed to determine the genetic diversity among the 38 grapevine cultivars, comprising 36 autochthonous cultivars from the Aegean region and two reference cultivars in this study. Eleven SSR primers were used which generated a total of 37 alleles. All loci were polymorphic. The microsatellite markers used in our study have been proven as very useful for cultivar identification. Genetic similarities based on Dice coefficients ranged between 0.296 and 0.882 (Table 2). The most informative locus was VVMD7 based on the number of alleles generated (6 alleles) and probability of identity values whereas the least informative locus was VVS2 which generated only 2 alleles.

To elucidate the genetic relationship among the cultivars a UPGMA dendrogram was generated (Figure 1). Two European cultivars Cabernet Sauvignon and Merlot formed an out group that they were separated from the Turkish local cultivars which clearly shows the genetic differences. Local cultivars were classified into two main groups. In the first one, Sultan Dimriti, Veyis and Güvercin

Table 1- Basic descriptive characteristic of 36 autochthonous and 2 standard grapevine cultivars

Çizelge 1- Otuzaltı yerel ve 2 standart üzüm çeşidinin temel tanımlayıcı karakterleri

No	*Cultivar*	*Utility*	*Ripening**	*Berry colour*	*Cluster characteristics (form)*	*Location of collection*
C	Cabernet Sauvignon	wine	L	black with heavy blue gray bloom	long conical-cylindrical	Manisa
M	Merlot	wine	M	blue-black	pyramidale-cylindrical	Manisa
1	Sultan 7	raisin	M	green-yellow	winged long cylindrical	Manisa
2	Sultan Dimriti	table	ML	dark violet	winged conical	Denizli
3	Pembe Üzüm	table	ML	pink	conical	Aydın
4	Pembe Salman	table	ML	pink	conical	Aydın
5	Kırmızı Razakı	table	ML	pink	winged conical	Aydın
6	Bir Çekirdekli	table	M	green-yellow	winged	Kütahya
7	Rezina	table	M	green-yellow	winged	Manisa
8	Kara Üzüm Dimriti	table	M	dark violet	winged conical	Manisa
9	Yerli Dimrit	table	M	dark violet	winged conical	Manisa
10	Kara Dimrit	table-raisin	M	reddish purple	winged cylindrical	Afyon
11	Corint	raisin	M	reddish black	winged cylindrical	Denizli
12	Gelin Üzümü	table- grape juice	M	reddish grey	conical	Manisa
13	Analı Kızlı	grape juice	ML	dark red-violet	conical	Aydın
14	Akdimrit	table	ML	green-yellow	cylindrical	Denizli
15	Dimrit	table	ML	dark violet	winged conical	Denizli
16	Köy Yeri	table-raisin	M	reddish grey	conical	Manisa
17	Gelin Üzümü	table- grape juice	M	reddish grey	conical	Afyon
18	Siyah Razakı	table	M	violet-black	winged conical	Kütahya
19	Parmak	table	M	black	cylindrical	Afyon
20	Eşek Memesi	table	ML	white	cylindrical	Denizli
21	Burdur Dimriti	table	M	dark red-violet	conical	Afyon
22	İnek Memesi	table	M	white	cylindrical	Manisa
23	Şam Üzümü	table	ML	white	conical	Manisa
24	Ufak Dimrit	table	ML	white	conical	Afyon
25	Foça Razakısı	table	ML	black	conical	Aydın
26	Kadın Parmağı	table	ML	green-yellow	long cylindrical	Denizli
27	Çeşme Beyazı	table	M	green-yellow	cylindrical	İzmir
28	Erkenci Beyaz Üzümü	grape juice	M	green-yellow	conical	Kütahya
29	Salman	table	M	green-yellow	long cylindrical	Manisa
30	Pembe Çekirdeksiz	table	M	pink	winged cylindrical	Manisa
31	Siyah Çekirdeksiz	table	M	yellow-green	winged	Manisa
32	Devegözü	table	M	green-yellow	conical	Manisa
33	Beyaz Şam	table	M	yellow-green	winged	Manisa
34	Veyis	table	ML	red	conical	Afyon
35	Güvercin Gözü	table-grape juice	M	green-yellow	conical	Aydın
36	Tek Çekirdekli	table	M	white	cylindrical	Manisa

*, L, late season; M, mid season; ML, mid-late season

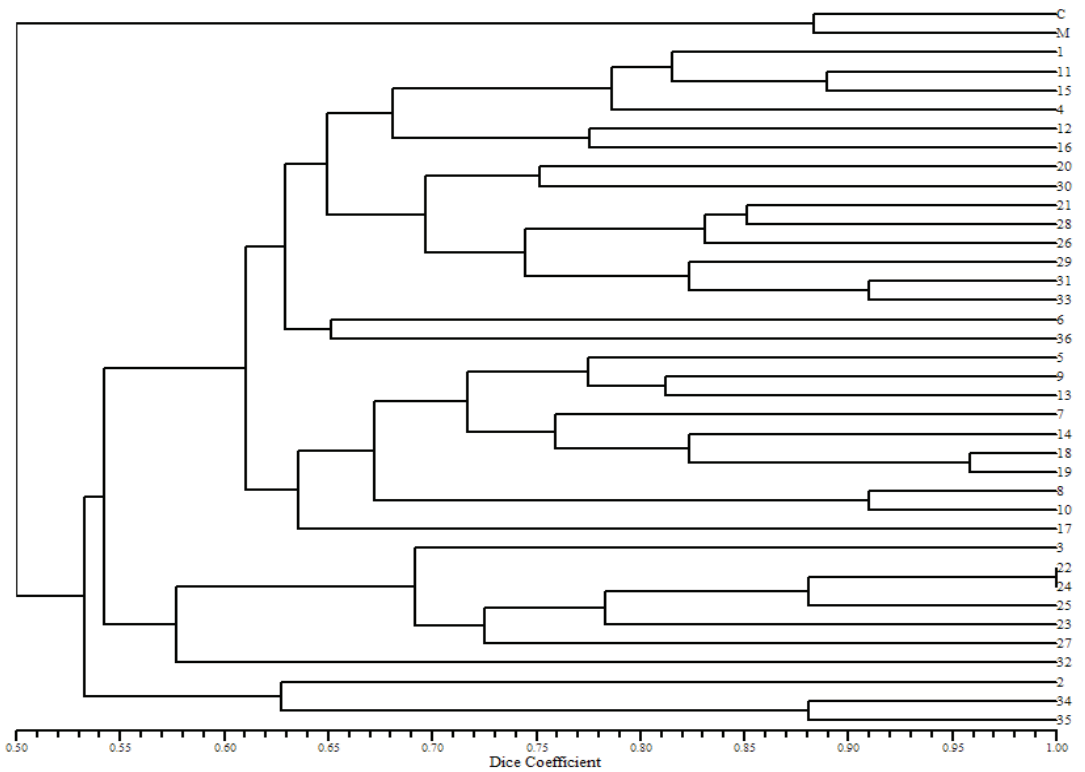

Figure 1- UPGMA dendrogram based on Dice genetic similarity coefficients for 36 autochthonous and 2 standard grapevine cultivars

Şekil 1- Dice genetik benzerlik katsayısına göre otuzaltı yerel ve 2 standart üzüm çeşidi için elde edilen UPGMA dendrogram (C, Cabernet Sauvignon; M, Merlot; 1, Sultan-7; 2, Sultan Dimriti; 3, Pembe Üzüm; 4, Pembe Salman; 5, Kırmızı Razakı; 6, Bir Çekirdekli; 7, Rezina; 8, Kara Üzüm Dimriti; 9, Yerli Dimrit; 10, Kara Dimrit; 11, Corint; 12, Gelin Üzümü; 13, Analı Kızlı; 14, Akdimrit; 15, Dimrit; 16, Köy Yeri; 17, Gelin Üzümü; 18, Siyah Razakı; 19, Parmak; 20, Eşek Memesi; 21, Burdur Dimriti; 22, İnek Memesi; 23, Şam Üzümü; 24, Ufak Dimrit; 25, Foça Razakısı; 26, Kadın Parmağı; 27, Çeşme Beyazı; 28, Erkenci Beyaz Üzümü; 29, Salman; 30, Pembe Çekirdeksiz; 31, Siyah Çekirdeksiz; 32, Devegözü; 33, Beyaz Şam; 34, Veyis; 35, Güvercin Gözü; 36, Tek Çekirdekli).

Gözü were clustered separately from the remaining cultivars. The second one was the largest (33 cultivars) and the most complex cluster. The high level of within-group variation and the simple genetic structure observed in the dendrogram suggested a complex history of development of cultivars in the Aegean region. Dice genetic similarity coefficients showed that the cultivars Siyah Razakı and Parmak (0.96), Kara Üzüm Dimriti and Kara Dimrit (0.91), Siyah Çekirdeksiz and Beyaz Şam (0.91), Corint and Dimrit (0.90), Veyis and Güvercin Gözü (0.88), İnek Memesi, Ufak Dimrit and Foça Karası (0.88) were the most similar. We found a relatively high level of genetic diversity among the cultivars in comparison with similar studies (Yüksel 2008).

İnek Memesi and Ufak Dimrit cultivars were placed in group 2 which were synonyms (Figure 1 and Table 2). These cultivars have multiple names

Table 2- Dice genetic similarity coefficients for 36 autochthonous and 2 standard grapevine cultivars

Çizelge 2- Otuzaltı yerel ve 2 standart üzüm çeşidi için Dice genetik benzerlik katsayısı

Cultivar	*C*	*M*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*	*31*	*32*	*33*	*34*	*35*	*36*
C	1.000																																					
M	0.882	1.000																																				
1	0.500	0.579	1.000																																			
2	0.483	0.452	0.667	1.000																																		
3	0.438	0.412	0.556	0.483	1.000																																	
4	0.424	0.514	0.757	0.600	0.545	1.000																																
5	0.514	0.486	0.615	0.625	0.514	0.667	1.000																															
6	0.606	0.629	0.649	0.600	0.424	0.529	0.611	1.000																														
7	0.486	0.564	0.634	0.412	0.595	0.579	0.700	0.684	1.000																													
8	0.606	0.686	0.649	0.600	0.364	0.588	0.722	0.647	0.632	1.000																												
9	0.471	0.556	0.579	0.516	0.529	0.629	0.757	0.571	0.667	0.686	1.000																											
10	0.563	0.647	0.722	0.621	0.313	0.667	0.686	0.667	0.649	0.910	0.647	1.000																										
11	0.571	0.649	0.872	0.750	0.514	0.833	0.632	0.667	0.600	0.667	0.649	0.743	1.000																									
12	0.546	0.629	0.703	0.600	0.485	0.706	0.500	0.647	0.632	0.530	0.571	0.606	0.833	1.000																								
13	0.514	0.541	0.667	0.625	0.400	0.611	0.789	0.611	0.650	0.667	0.811	0.686	0.737	0.611	1.000																							
14	0.486	0.564	0.683	0.529	0.486	0.684	0.650	0.737	0.762	0.684	0.667	0.757	0.750	0.632	0.750	1.000																						
15	0.424	0.514	0.757	0.667	0.424	0.765	0.556	0.706	0.526	0.588	0.514	0.667	0.889	0.765	0.667	0.789	1.000																					
16	0.467	0.500	0.529	0.593	0.533	0.645	0.485	0.516	0.514	0.516	0.500	0.533	0.606	0.774	0.485	0.571	0.645	1.000																				
17	0.375	0.412	0.556	0.552	0.688	0.606	0.571	0.485	0.595	0.606	0.647	0.563	0.629	0.606	0.514	0.703	0.606	0.733	1.000																			
18	0.500	0.524	0.591	0.432	0.550	0.585	0.744	0.634	0.756	0.634	0.714	0.600	0.651	0.585	0.744	0.844	0.683	0.579	0.750	1.000																		
19	0.500	0.524	0.591	0.486	0.550	0.634	0.791	0.585	0.756	0.683	0.762	0.650	0.651	0.537	0.744	0.800	0.634	0.579	0.750	0.958	1.000																	
20	0.414	0.387	0.667	0.615	0.621	0.600	0.500	0.533	0.353	0.467	0.387	0.483	0.625	0.467	0.438	0.471	0.600	0.519	0.552	0.486	0.486	1.000																
21	0.486	0.564	0.732	0.647	0.486	0.737	0.550	0.684	0.571	0.632	0.564	0.595	0.800	0.632	0.700	0.714	0.842	0.571	0.541	0.667	0.667	0.647	1.000															
22	0.500	0.400	0.625	0.560	0.714	0.483	0.452	0.621	0.424	0.414	0.333	0.357	0.581	0.414	0.387	0.485	0.621	0.462	0.500	0.500	0.444	0.800	0.667	1.000														
23	0.387	0.364	0.629	0.500	0.774	0.500	0.471	0.563	0.611	0.438	0.424	0.387	0.588	0.500	0.471	0.611	0.625	0.552	0.710	0.667	0.615	0.643	0.667	0.815	1.000													
24	0.500	0.400	0.625	0.560	0.714	0.483	0.452	0.621	0.424	0.414	0.333	0.357	0.581	0.414	0.387	0.489	0.621	0.462	0.500	0.500	0.444	0.800	0.667	1.000	0.815	1.000												
25	0.621	0.516	0.606	0.462	0.621	0.467	0.438	0.600	0.471	0.533	0.323	0.483	0.563	0.467	0.375	0.471	0.600	0.444	0.414	0.486	0.432	0.692	0.647	0.880	0.714	0.880	1.000											
26	0.486	0.564	0.634	0.529	0.432	0.579	0.750	0.737	0.667	0.789	0.718	0.703	0.650	0.526	0.800	0.762	0.684	0.514	0.595	0.800	0.800	0.588	0.810	0.545	0.611	0.545	0.530	1.000										
27	0.500	0.529	0.556	0.552	0.625	0.485	0.400	0.667	0.541	0.485	0.412	0.438	0.629	0.545	0.457	0.649	0.667	0.533	0.625	0.650	0.600	0.621	0.703	0.714	0.774	0.714	0.690	0.595	1.000									
28	0.514	0.541	0.667	0.625	0.571	0.667	0.684	0.611	0.600	0.611	0.649	0.571	0.737	0.611	0.789	0.650	0.722	0.606	0.571	0.744	0.744	0.688	0.850	0.645	0.706	0.645	0.625	0.850	0.686	1.000								
29	0.556	0.579	0.600	0.424	0.500	0.595	0.667	0.703	0.585	0.649	0.684	0.611	0.615	0.595	0.667	0.683	0.649	0.647	0.667	0.773	0.773	0.667	0.683	0.563	0.571	0.563	0.545	0.829	0.611	0.718	1.000							
30	0.457	0.486	0.718	0.563	0.514	0.667	0.421	0.556	0.450	0.556	0.541	0.571	0.684	0.556	0.526	0.600	0.667	0.606	0.629	0.605	0.605	0.750	0.800	0.645	0.647	0.645	0.625	0.650	0.686	0.737	0.667	1.000						
31	0.485	0.514	0.595	0.533	0.485	0.647	0.500	0.647	0.474	0.471	0.571	0.485	0.667	0.706	0.611	0.579	0.706	0.774	0.606	0.634	0.634	0.667	0.737	0.552	0.625	0.552	0.533	0.684	0.667	0.778	0.811	0.722	1.000					
32	0.296	0.276	0.645	0.417	0.667	0.643	0.467	0.357	0.438	0.286	0.552	0.370	0.600	0.500	0.467	0.375	0.500	0.480	0.519	0.457	0.514	0.667	0.500	0.609	0.615	0.609	0.500	0.438	0.4444	0.600	0.581	0.600	0.643	1.000				
33	0.438	0.471	0.611	0.483	0.563	0.667	0.514	0.545	0.432	0.485	0.588	0.500	0.686	0.606	0.629	0.595	0.727	0.667	0.625	0.650	0.650	0.759	0.757	0.643	0.645	0.643	0.621	0.703	0.688	0.800	0.833	0.743	0.910	0.741	1.000			
34	0.444	0.345	0.516	0.583	0.370	0.429	0.400	0.500	0.250	0.357	0.276	0.444	0.533	0.429	0.467	0.438	0.571	0.480	0.296	0.400	0.400	0.667	0.563	0.609	0.462	0.609	0.583	0.500	0.519	0.600	0.516	0.533	0.643	0.545	0.667	1.000		
35	0.467	0.438	0.588	0.667	0.400	0.452	0.424	0.516	0.286	0.452	0.313	0.467	0.606	0.516	0.545	0.457	0.645	0.571	0.4000	0.474	0.474	0.741	0.686	0.615	0.483	0.615	0.593	0.571	0.600	0.667	0.588	0.606	0.710	0.480	0.733	0.880	1.000	
36	0.500	0.474	0.550	0.545	0.611	0.595	0.513	0.649	0.634	0.432	0.526	0.444	0.616	0.595	0.564	0.683	0.649	0.647	0.611	0.727	0.727	0.545	0.683	0.563	0.629	0.563	0.545	0.585	0.722	0.718	0.600	0.615	0.757	0.516	0.667	0.581	0.647	1.000

associated with the area of their origin**.** Synonyms detected in this study were lower than those previously reported (Ergül et al 2006; Karataş et al 2007; Şelli et al 2007; Tangolar et al 2009). Genotypes grown under the same name of Dimrit in three different areas (Afyon, Denizli and Manisa provinces) were found in the two main groups on the dendogram. Sultan Dimriti (0.63) was clustered separately from the remaining other Dimrit genotypes. The second main cluster was further divided into two subgroups. The first subgroup included Ufak Dimrit (0.88). The second group was further divided into two subgroups comprising Kara Üzüm Dimriti (0.91), Yerli Dimrit (0.81), Kara Dimrit (0.91) and Akdimrit (0.82), the second subgroup in the second main cluster comprised Dimrit (0.88) and Burdur Dimriti (0.84). Our results showed that Dimrit genotypes from different locations were different from each other which indicate an important genetic variation which are good example of homonymous cultivars. They have been cultivated in different environments for long times, and those transferred to the Manisa Viticulture and Research Station could be inappropriately named. Changes in genetic background of these cultivars probably are caused by somatic mutations which could be due to effects of continuous vegetative reproduction and environmental factors. Turkey is a very rich in terms of homonymous grape cultivars. Because they have long history of cultivation since ancient tradition of grape cultivation in Anatolia which began approximately 7000–8000 years ago (Karataş et al 2007; Yüksel 2008).

4. Conclusions

The results showed that the gene pool of cultivated local grapes surveyed in the Aegean region has a significant amount of genetic variation. SSR analysis revealed better characterization of the grape germplasm grown in the region and would aid future germplasm management of cultivar numbers and breeding efforts. Characterization of genotypes will also contribute to the other viticulture activities such as plant propagation and nursery management.

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