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Genetic characterization of grape (*Vitis vinifera* L.) germplasm from Southeast Anatolia by SSR markers

Y. Boz[1)], M. Bakır[2)], B. P. Çelikkol[2)], K. Kazan[3)], F. Yilmaz[2)], B. Çakır[4)], Ş. Aslantaş[2)], G. Söylemezoğlu[5)], A. S. Yaşasin[1)], C. Özer[1)], H. Çelik[5)] and A. Ergül[2)]

[1)] Ministry of Agriculture and Rural Affairs, Institute of Viticulture, Tekirdağ, Turkey
[2)] Ankara University, Biotechnology Institute, Ankara, Turkey
[3)] Commonwealth Scientific and Industrial Research Organization (CSIRO) Plant Industry, Queensland Bioscience Precinct, St. Lucia, Queensland, Australia
[4)] Ege University, Faculty of Agriculture, Department of Horticulture, İzmir, Turkey
[5)] Ankara University, Faculty of Agriculture, Department of Horticulture, Ankara, Turkey

Summary

Southeast Anatolia is located in close proximity to the center of origin of grapes and is an important grape producing area of Turkey. The important location of this region for grape genetic diversity together with its diverse ecological conditions may have led to the development of grape germplasm that is unique to this region. However, so far little has been done to genetically analyze this grape germplasm. In this study, we genetically analyzed 55 grape cultivars originating from six different provinces of this region using 14 simple sequence repeat (SSR) loci and a number of ampeolographic characteristics. Based on these analyses, one case of synonymous and four cases of homonymous grape cultivars were identified. The contribution of our results to better characterization of the grape germplasm of the region as well as future germplasm management and breeding efforts is discussed.

K e y w o r d s : *Vitis vinifera* L., SSR, Southeast Anatolia.

Introduction

Southeast Anatolia is a significant grape (*Vitis vinifera* L.) growing region of Turkey, producing 540,899 tonnes of fresh grape annually (Anonymous 2007). Diverse ecological conditions that exist within Southeast Anatolia make the cultivation of both early- and late-pipening grape cultivars possible. Grapes produced in this region are mostly consumed as table grapes with relatively small amounts used in wine-making and in snack food industries. Gaziantep, Diyarbakır, and Şanlıurfa provinces of this region are the major viticulture areas, followed by Mardin, Adıyaman and Siirt provinces. The region also contains a rich grape germplasm. The existence of wild grape populations in the region together with recent archeological findings (McGovern 2003) suggest that viticulture has long been known in the region.

Despite the importance of this region as a local center of grape diversity, so far little has been done to characterize the grape cultivars grown in this region. In a previous study, homonyms of a few cultivars widely grown in Gaziantep and Şanlıurfa were identified using molecular techniques (Karataş *et al.* 2007, Karataş and Ağaoğlu 2008). However, the genetic relatedness of cultivars originating from different provinces of this region with different ecological conditions has not been studied using molecular markers in a single study. Better characterization of the grape germplasm of this region would aid breeding and germplasm management activities.

The objective of this study was to genetically characterize nearly all known grape cultivars of Southeast Anatolia. For this purpose, 55 grape cultivars, which were included in the "National Grapevine Germplasm Vineyard*"* as a representative of the regional grape genetic diversity, were analyzed using 14 Simple Sequence Repeat (SSR) primer pairs. The genetic relationships of grape cultivars originating from six different provinces of the region were determined and synonymous and homonymous cultivars identified. In addition, for the first time, ampelographic characteristics of these grape cultivars were documented. The results reported here would be useful in grape breeding as well as in studies on genetic relatedness.

Material and Methods

P l a n t m a t e r i a l : A total of 55 grape cultivars were analyzed in this study. These grape cultivars were obtained from the National Grapevine Germplasm Vineyard at the Institute of Viticulture in Tekirdağ, Turkey. Original locations and some ampelographic characteristics of the cultivars studied are given in Tab. 1. Three reference cultivars ('Cabernet-Sauvignon', 'Merlot' and 'Pinot Noir') present in the collection were included in the analysis.

D N A i s o l a t i o n : DNA was extracted from the grape leaf tissue as described by Lefort *et al*. (1998). 100 mg of young leaves were ground to a fine powder in liquid nitrogen and homogenized. The powder was transferred to a new 2 ml polypropylene tube and 1 ml of DNA extraction buffer (50 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 0.7 mM NaCl, 1 % w/v CTAB (hexadecytrimethylammonium bromide), 2 % (w/v) PVP 40 and 10 µl of

Correspondence to: Prof. Dr. A. Ergül, Ankara University, Institute of Biotechnology, Central Laboratory, 06100 Tandogan-Ankara, Turkey. Fax +90-312-222-5872. E-mail: ergul@agri.ankara.edu.tr

Table 1

Ampelographic characteristics and original collection regions of the grape cultivars used in this study

No	Cultivar Name	Provinces (town/city)	Cluster Form	Berry Form	Berry Color	Flavor	Seed no.	Ripening
1	Kızıl Üzüm (Kızıl Fertik)	Gölbaşı/Adıyaman	W. Conical	Ovoid	Black	Sweet	2-3	Late September
2	Kuraş	Gölbaşı/Adıyaman	W. Conical	Round	White	Sweet	2-3	Early September
3	Mazrune (Mazirone)	Kahta/Adıyaman	W. Conical	Round	White	Sweet	2-4	Early September
4	Samrı	Besni/Adıyaman	W. Conical	Ovoid	White	Sweet	1-3	Early September
5	Göğ Kuraş	Gölbaşı/Adıyaman	Conical	Round	White	Sweet	2-3	Late August
6	Şeker Ufağı	Gölbaşı/Adıyaman	Conical	Ovoid	White	Sweet	2	Late August
7	Kızlar Tahtası	Besni/Adıyaman	W. Conical	Ellipsoidal	White	Sweet	2-4	Early September
8	Peygamber (Besni)	Gölbaşı/Adıyaman	W. Conical	L. Ovoid	White	Sweet	2-3	Mid-August
9	Ballıboz	Gölbaşı/Adıyaman	Cylindrical	Ovoid	White	Sweet	2-3	Mid-September
10	Samrı	Besni/Adıyaman	W. Conical	Round	White	Sweet	2-4	Mid-September
11	Gülgülü	-/Adıyaman	W. Conical	Ellipsoidal	Red	Sweet	2-3	Late August
12	Yuvarlak Beyaz	-/Adıyaman	W. Conical	Ellipsoidal	White	Sweet	2-3	Late August
13	Serpene Kıran	Gölbaşı/Adıyaman	Conical	Round	White	Sweet	1-2	Early September
14	Çınar Yaprağı	Gölbaşı/Adıyaman	W. Conical	Ovoid	White	Sweet	2	Early September
15	Avi	Kahta/Adıyaman	Conical	Ovoid	White	Sweet	2-3	Late August
16	Kahti Göğ	Gölbaşı/Adıyaman	W. Conical	Round	White	Sweet	2-3	Mid-September
17	Kara Tümbü	Gölbaşı/Adıyaman	W. Conical	Round	Black	Sweet	2	Mid-September
18	Şekeri	Ergani/Diyarbakır	Conical	Round	White	Sweet	3	Early August
19	Gergeri	Center/Diyarbakır	Conical	Ellipsoidal	White	Sweet	2-3	Mid-September
20	Mikeri	Center/Diyarbakır	-	-	Black	-	-	Mid-August
21	Abdullah (Apo)	Center/Diyarbakır	Conical	Round	Red	Sweet	2-3	Early September
22	Muhammediye (Mor üzüm)	Ergani/Diyarbakır	Conical	Ellipsoidal	Black	Sweet	1-2	Late July
23	Vanki (Ceyn Vagi)	Ergani/Diyarbakır	Cylindrical	L. Elipsoidal	White	Sweet	2-3	Mid-July
24	Unknown	-/Diyarbakır	Conical	Round	Pink	Sweet	1-2	Mid-September
25	Tahannebi (Mehmet Yakup üzümü)	Ergani/Diyarbakır	W. Conical	Ellipsoidal	White	Sweet	1-2	Mid-June
26	Künefi	Kilis/Gaziantep	W. Conical	Ovoid	Red	Sweet	2-3	Mid-September
27	Rumi	Kilis/Gaziantep	Conical	Round	White	Sweet	2-4	Mid-September
28	Tusboğa Kabarcığı	Kilis/Gaziantep	W. Conical	Round	White	Sweet	3-4	Mid-September
29	Dımışkı	Kilis/Gaziantep	Conical	Ovoid	White	Neutral	2-3	Mid-September
30	Oğlak Karası(Deve Gözü)	Kilis/Gaziantep	W. Conical	Ovoid	Black	Sweet	1-2	Mid-August
31	Üvezi	-/Gaziantep	W. Conical	Ellipsoidal	White	Sweet	3-4	Early September
32	Hönüsü	Kilis/Gaziantep	W. Conical	Cylindrical	Red	Sweet	2-3	Early October
33	Haseni	Savur/Mardin	W. Conical	Round	White	Sweet	2-3	Late August
34	Musabbık	Gercüş/Mardin	W. Conical	Round	White	Sweet	2-3	Mid-July
35	Tayifi	Gercüş/Mardin	L. Cylindrical	Ovoid	Black	Sweet	2-3	Early August
36	Aftık (Hılsık Deyvani)	Savur/Mardin	Cylindrical	Elipsoidal	White	Sweet	2-3	Late August
37	Bizani	Savur/Mardin	W. Conical	Ovoid	White	Sweet	2-3	Mid-August
38	Siyah Aftık (Siyah Deyvani/ Hılsı Kireş)	Savur/Mardin	Cylindrical	Elipsoidal	Black	Sweet	2-3	Early August
39	Reşe Drejik (Siyah Hatun Parmağı)	Gercüş/Mardin	W. Conical	L. Elipsoidal	Black	Sweet	2-3	Mid-August
40	Sıtvi (Kışlık Üzüm)	Savur/Mardin	Conical	Ovoid	White	Sweet	2-3	Mid-September
41	Vırdani (Harmani)	Savur/Mardin	L. Cylindrical	Ovoid	Red-purple	Sweet	2-3	Early September
42	Zeyti	Savur/Mardin	W. Conical	Round	White	Neutral	3-4	Mid-August
43	Ergit (Asmalı)	Bilgi/Siirt	W. Conical	Round	White	Sweet	2-3	Mid-September
44	Şuaybi	Aydınlar/Siirt	Conical	Elipsoidal	Black	Neutral	2	Early September
45	Hasani	Center/Siirt	Conical	Ovoid	White	Sweet	2-3	Early September
46	Reşmen	-/Siirt	W. Conical	Ovoid	Black	Sweet	3	Late August
47	Unknown	-/Siirt	Conical	Round	Black	Sweet	2-3	Early September
48	Kaysı	Center/Şanlıurfa	Conical	Ovoid	White	Sweet	2-3	Mid-June
49	Ruhali (Küllahi)	Hilvan/Şanlıurfa	W. Conical	Elipsoidal	White	Sweet	2-3	Mid-September
50	Çiloreş	Hilvan/Şanlıurfa	Conical	Elipsoidal	White	Sweet	2-3	Mid-September
51	Çilorut	Center/Şanlıurfa	W. Conical	Ovoid	White	Sweet	2-3	Mid-August
52	Mazrune (Siverek üzümü/Batık Kabarcığı)	Hilvan/Şanlıurfa	W. Conical	Round	White	Sweet	2-3	Mid-September
53	Tilgören	Hilvan/Şanlıurfa	Cylindrical	Round	Black	Sweet	2-4	Early September
54	Simore	Hilvan/Şanlıurfa	Conical	Elipsoidal	White	Sweet	2-3	Early September
55	Zerik	Hilvan/Şanlıurfa	Cylindrical	Round	White	Sweet	2-3	Late August

2-mercaptoethanol (1 % final concentration) added. The mixture was vortexed for 5 seconds and then incubated for 15 min at 65 °C in a water-bath. After incubation, an equal volume of chloroform/isoamyl alcohol (24:1) was added and the phases were separated by centrifugation at 16,000 g for 10 min. The aqueous layer was collected and 0.54 volume of cold isopropanol (-20 °C) added to precipitate the DNA. The DNA pellet was obtained after centrifugation at 16,000 g for 10 min and resuspended in 100 µl TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) containing 15 µg ml^{-1} RNAse A and incubated for 30 min at 37 °C. Proteins were removed by adding 50 µl 7.5 M ammonium acetate, followed by centrifugation at 16,000 g for 10 min. DNA in the supernatant was precipitated with a 0.54 volume of cold isopropanol, the pellet was dried at room temperature, resuspended in 100 µl TE and stored at 4 °C. The DNA concentration was estimated spectrophotometrically and the DNA quality was checked by agarose gel electrophoresis.

SSR and genetic analysis: Fourteen SSR markers, namely VVS2 (Thomas and Scott 1993), VVMD5, VVMD7, VVMD24, VVMD27, VVMD28,

VVMD31 (Bowers *et al*. 1996, 1999), ZAG62, ZAG79, ZAG83 (Sefc *et al.* 1999), VMC2h4, VMC2c3 (Goto-Yamamoto *et al.* 2006) and VVIh54, VVIb01 (Merdinoglu *et al.* 2005), were used in this study. Six of these loci belong to the so called "core SSR marker set" that allows direct comparisons of allele sizes from different grape cultivars analyzed in different studies (This *et al.* 2004). PCR amplifications were performed in a reaction volume of 10 µl containing 15 ng of DNA, 5 pmol of each primer, 0.5 mM dNTP, 0.5 units GoTaq DNA Polymerase (Promega, Madison, WI) that includes 1.5 mM $MgCl_2$. Forward primers of each primer pair were labeled with WellRED fluorescent dyes D2 (black), D3 (green) and D4 (blue) (Proligo, Paris, France). PCR conditions had an initial cycle of 3 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 55-60 °C and 2 min at 72 °C with a final extension at 72 °C for 10 min. PCR products were diluted with SLS (sample loading solution) in certain proportions according to the fluorescent dyes used in labeling, followed by the addition of Genomelab DNA Standard Kit-400 and electrophoresed in CEQ 8800XL capillary DNA analysis system (Beckman Coulter, Fullerton, CA). Allele sizes were determined for each SSR locus using a Beckman CEQ fragment analysis software. In each run, 'Cabernet Sauvignon', 'Merlot' and 'Pinot Noir' were included as reference cultivars. These analyses were repeated at least twice to ensure reproducibility of the results.

Fifty-five grape cultivars from all six provinces of the region were surveyed with the fourteen SSR markers given above: Adıyaman (17 cultivars), Diyarbakır (8 cultivars), Gaziantep (7 cultivars), Mardin (10 cultivars), Siirt (5 cultivars) and Şanlıurfa (8 cultivars). Factorial Correspondence Analysis using the Genetix4 software (Belkhir *et al.* 1996-98) was also performed to determine the presence of any province-dependent structuring of the grape cultivars studied. Possible gene flows among accessions of different provinces were estimated and linkage disequilibrium tested for each loci by Genetix 4.05 to determine if there is any significant association among alleles of different locus. A neighbour joining tree was constructed from Nei's genetic distance (Nei 1972) using NTSYS-pc (Rohlf 2004).

Number of alleles (n), allele frequency, expected (He) and observed (Ho) heterozygosity, estimated frequency of null alleles (r) and probability of identity (PI) were calculated for each locus using the program "IDENTITY" 1.0 (Wagner and Sefc 1999) according to Paetkau *et al*. (1995). The software "IDENTITY" was also used to detect identical cultivars. Proportion of shared alleles was calculated by using ps (option 1-(ps)) as described by Bowcock *et al*. (1994) as genetic dissimilarity by the program Microsat (version 1.5) (Minch *et al*. 1995). These data were then converted into a similarity matrix to determine genetic similarity among grape cultivars.

Results

S S R a n a l y s e s : In this study, we screened fifty-five grape cultivars from Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt and Şanlıurfa provinces within the Southeast Anatolian region of Turkey using 14 SSR markers. The three reference cultivars, 'Cabernet Sauvignon', 'Merlot' and 'Pinot Noir', were also studied (Tab. 2). Specific allele sizes revealed by these primers are presented in Tab. 2. A total of 119 alleles were detected at these 14 SSR loci, with an average allele number of 8.500 (Tab. 3). The most informative loci was VVS2 with thirteen alleles while VVIb01 and VMC2c3 with five alleles and ZAG83 with six alleles were found to be the least informative loci (Tab. 3). The mean observed heterozygosity (Ho) and the expected heterozygosity (He) values were 0.714 and 0.752, respectively. The highest level of observed heterozygosity (0.862) was detected at VVS2 while the lowest (0.534) was at ZAG83. The expected heterozygosity ranged from 0.53 for VVIb01 to 0.849 for VMC2h4 and VVMD5.

G e n e t i c r e l a t i o n s h i p s a m o n g g r a p e s f r o m d i f f e r e n t p r o v i n c e s : The pairwise F_{ST} values among grapes from different provinces were calculated (Tab. 4). Based on the differentiation values and the phylogenetic tree constructed by neighbor joining analysis (data not shown), only the Mardin province was significantly different from those of the other five provinces (data not shown). The gene flow (Nm) values between Mardin and each of the remaining provinces were also low (Tab. 5). In contrast, there appears to be higher levels of gene flow among the remaining provinces. Therefore, based on Nm values, other provinces could not be clearly distinguishable. Several significant ($P < 0.05$) linkage disequilibriums were detected among allele pairs at different loci. Mardin and Siirt provinces have the highest (52 pairs in 14 loci) and the lowest number of significant pairs (1 pair in 14 loci).

Discussion

S S R a n a l y s e s : SSR or microsatellite markers have many advantages over most other DNA markers as they are highly polymorphic, show a codominant mode of inheritance, and allow simple data interpretation (Thomas *et al.* 1994). In this study, we selected 14 SSR markers that are commonly used in *V. vinifera* L. for germplasm characterization, variety and clone identification and parentage analysis (Bowers *et al.* 1996, 1999, Sefc *et al*. 2000, Fatahi *et al.* 2003, Aradhya *et al.* 2003, Ibáñez *et al*. 2003, Merdinoglu *et al.* 2005, Costantini *et al.* 2005, Martinez *et al.* 2006, Gök Tangolar *et al.* 2009, Zoghlami *et al.* 2009). The average number of alleles found in the present work was comparable to those reported in other studies on grapes (Dangl *et al.* 2001, Costantini *et al*. 2005). However, using a smaller set of SSR loci (6 loci), Karataş *et al.* (2007) previously reported higher average allele numbers (14.6) in 16 grape cultivars from Gaziantep and Şanlıurfa than those found in the present work. In their report, these authors have characterized some of the similarly-named cultivars such as 'Hönüsü', 'Çilorut', 'Dımışkı', 'Çiloreş', 'Hatunparmağı', 'Serpenekıran', 'Gülgülü', 'Muhammadiye', which are also used in our study. However, because no accession numbers were given for the grape cultivars used by Karataş *et al.* (2007), we were not able to compare their

Table 2

Allele sizes (bp) of grape cultivars at 14 SSR loci. Allele sizes of the reference cultivars,
CS: Cabernet-Sauvignon, M: Merlot and PN: Pinot Noir (number 56, 57, and 58 respectively) are shown in bold

No.	ZAG79		VVIh54		VVMD24		VVMD7		VVMD28		VVMD27		VMC2h4		VVIbO1		ZAG83		VVS2		VVMD5		ZAG62		VVMD31		VMC2c3	
1	248	248	138	164	207	211	242	246	233	233	175	183	204	218	292	296	185	191	133	135	233	235	188	192	209	209	163	167
2	246	246	150	176	207	215	246	252	257	257	175	185	202	214	292	296	187	191	133	143	233	233	192	204	209	211	163	163
3	242	248	150	150	207	209	246	248	257	281	179	195	200	214	292	316	191	191	133	143	225	245	192	200	209	209	163	189
4	242	242	150	150	209	215	232	248	257	257	195	195	202	214	292	292	185	191	133	143	235	245	200	204	209	209	163	189
5	246	248	150	166	207	215	252	254	233	257	177	179	200	214	292	296	187	191	143	155	225	233	202	202	211	215	163	163
6	246	248	150	176	217	217	238	252	257	281	179	185	206	214	292	292	191	191	143	155	233	237	196	204	209	211	163	189
7	242	256	150	164	207	209	248	248	235	257	175	195	200	206	296	316	185	191	133	133	225	225	200	204	209	213	167	189
8	256	256	150	166	207	217	246	246	233	281	175	195	200	206	292	292	185	191	141	151	231	237	192	204	209	215	163	177
9	242	246	164	176	211	217	248	252	257	257	177	195	206	214	292	292	185	187	139	143	233	245	202	204	195	211	163	167
10	248	248	138	166	209	211	242	248	233	257	175	185	204	206	292	296	185	191	133	135	235	235	204	204	209	209	163	189
11	246	250	164	174	207	217	242	248	233	257	179	185	198	218	296	296	185	197	137	143	235	239	188	200	209	211	163	167
12	250	250	166	166	207	217	246	248	233	257	185	195	198	206	296	296	191	191	151	155	227	233	200	204	211	215	163	167
13	246	248	150	150	207	207	246	252	257	281	177	179	202	202	292	296	191	191	133	133	233	245	192	204	211	213	163	189
14	248	256	166	176	207	207	246	248	243	281	179	195	206	206	292	296	191	191	135	155	225	245	192	192	209	209	167	189
15	248	248	138	164	207	211	242	246	233	233	183	183	204	218	292	296	191	191	133	135	233	235	188	192	211	215	163	167
16	246	248	150	166	207	215	252	254	233	257	177	179	200	214	292	296	187	191	143	155	225	233	204	204	211	215	163	163
17	242	248	150	150	207	209	246	248	257	281	179	195	200	214	292	316	191	191	133	143	225	245	192	200	209	215	163	189
18	242	248	164	164	207	211	246	246	243	257	179	195	202	206	292	316	191	191	143	151	225	231	192	204	209	213	163	163
19	246	250	138	150	207	209	246	248	235	243	179	195	198	212	292	296	185	191	135	155	225	235	192	200	209	209	163	189
20	250	256	138	164	207	207	246	248	257	257	183	195	206	206	292	292	185	185	143	151	235	239	190	204	209	211	163	163
21	248	248	138	164	207	211	242	246	233	233	183	183	204	218	292	296	191	191	133	135	233	235	188	192	209	209	163	167
22	246	246	150	176	207	215	246	252	257	257	177	179	202	214	292	296	187	191	133	143	233	233	192	204	209	211	163	163
23	256	256	150	166	207	217	246	246	233	281	195	195	200	206	292	292	185	191	141	151	229	235	192	204	209	215	163	177
24	242	256	138	138	207	211	246	246	257	277	195	195	202	214	296	296	185	197	135	135	231	239	200	204	209	213	167	167
25	256	256	164	176	209	211	246	246	257	257	195	195	206	206	292	296	191	191	133	155	233	237	192	204	209	209	167	189
26	248	248	138	164	207	211	242	246	233	233	183	183	204	218	292	296	191	191	133	135	233	235	188	192	209	209	163	191
27	246	256	166	176	207	211	242	248	233	257	175	195	200	206	292	308	185	185	131	133	233	245	188	188	195	213	163	191
28	246	246	150	176	207	217	246	252	257	257	177	179	202	214	292	296	187	191	133	143	233	239	192	204	209	211	163	167
29	256	256	158	164	211	211	238	248	243	257	195	195	198	214	292	292	191	191	133	149	225	231	188	204	213	213	167	177
30	250	250	138	176	207	217	248	248	235	243	183	195	206	206	292	296	185	191	133	141	235	237	188	200	209	209	163	163
31	248	256	166	166	217	235	246	246	243	281	179	195	206	212	292	296	185	191	143	155	225	237	192	192	195	209	163	167
32	256	258	138	158	207	217	238	252	243	243	181	195	198	204	292	292	185	191	143	149	227	231	188	188	203	209	177	191
33	246	248	138	166	207	207	238	248	271	281	179	179	206	214	292	292	185	185	123	131	237	245	196	200	201	215	163	163
34	250	256	174	174	211	215	238	238	257	257	185	195	200	214	292	292	185	185	135	153	225	227	188	196	211	213	167	177
35	246	256	138	138	205	217	238	248	243	257	185	195	210	214	292	292	185	185	143	153	239	245	188	194	211	211	167	177
36	250	256	174	174	211	215	238	238	257	257	185	195	200	214	292	292	185	185	135	153	225	227	188	196	211	211	167	177
37	250	256	138	176	211	235	246	246	257	257	181	195	202	204	292	296	185	191	135	149	227	233	188	192	203	211	167	177
38	246	248	138	150	207	207	246	248	257	281	179	195	204	204	292	296	185	191	133	133	235	235	200	204	201	209	163	189
39	246	256	138	138	205	217	238	248	243	257	185	195	210	214	292	292	185	185	143	153	239	245	188	194	211	211	167	177
40	250	250	164	174	207	215	238	246	257	257	181	185	200	202	292	292	197	197	135	151	225	233	192	196	209	213	167	167
41	248	256	164	164	207	207	232	238	243	257	183	195	214	214	292	292	185	185	135	145	225	235	196	196	211	215	163	163

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Allele sizes (bp) of grape cultivars at 14 SSR loci. Allele sizes of the reference cultivars, CS: Cabernet-Sauvignon, M: Merlot and PN: Pinot Noir (number 56, 57, and 58 respectively) are shown in bold

No.	ZAG79		VVIh54		VVMD24		VVMD7		VVMD28		VVMD27		VMC2h4		VVIbO1		ZAG83		VVS2		VVMD5		ZAG62		VVMD31		VMC2c3	
1	248	248	138	164	207	211	242	246	233	233	175	183	204	218	292	296	185	191	133	135	233	235	188	192	209	209	163	167
2	246	246	150	176	207	215	246	252	257	257	175	185	202	214	292	296	187	191	133	143	233	233	192	204	209	211	163	163
3	242	248	150	150	207	209	246	248	257	281	179	195	200	214	292	316	191	191	133	143	225	245	192	200	209	209	163	189
4	242	242	150	150	209	215	232	248	257	257	195	195	202	214	292	292	185	191	133	143	235	245	200	204	209	209	163	189
5	246	248	150	166	207	215	252	254	233	257	177	179	200	214	292	296	187	191	143	155	225	233	202	202	211	215	163	163
6	246	248	150	176	217	217	238	252	257	281	179	185	206	214	292	292	191	191	143	155	233	237	196	204	209	211	163	189
7	242	256	150	164	207	209	248	248	235	257	175	195	200	206	296	316	185	191	133	133	225	225	200	204	209	213	167	189
8	256	256	150	166	207	217	246	246	233	281	175	195	200	206	292	292	185	191	141	151	231	237	192	204	209	215	163	177
9	242	246	164	176	211	217	248	252	257	257	177	195	206	214	292	292	185	187	139	143	233	245	202	204	195	211	163	167
10	248	248	138	166	209	211	242	248	233	257	175	185	204	206	292	296	185	191	133	135	235	235	204	204	209	209	163	189
11	246	250	164	174	207	217	242	248	233	257	179	185	198	218	296	296	185	197	137	143	235	239	188	200	209	211	163	167
12	250	250	166	166	207	217	246	248	233	257	185	195	198	206	296	296	191	191	151	155	227	233	200	204	211	215	163	167
13	246	248	150	150	207	207	246	252	257	281	177	179	202	202	292	296	191	191	133	133	233	245	192	204	211	213	163	189
14	248	256	166	176	207	207	246	248	243	281	179	195	206	206	292	296	191	191	135	155	225	245	192	192	209	209	167	189
15	248	248	138	164	207	211	242	246	233	233	183	183	204	218	292	296	191	191	133	135	233	235	188	192	211	215	163	167
16	246	248	150	166	207	215	252	254	233	257	177	179	200	214	292	296	187	191	143	155	225	233	204	204	211	215	163	163
17	242	248	150	150	207	209	246	248	257	281	179	195	200	214	292	316	191	191	133	143	225	245	192	200	209	215	163	189

Table 3

SSR loci, number of allele(n), expected heterozygosity (He), observed heterozygosity (Ho), probability of identity (PI) and null allele frequencies (r) for 55 grape cultivars analyzed at 14 SSR markers

Loci	n	He	Ho	PI	r
VVIh54	8	0.815	0.672	0.109	0.078
VVMD24	8	0.730	0.793	0.165	-0.036
VVMD7	7	0.775	0.741	0.150	0.019
VVMD28	10	0.720	0.672	0.176	0.027
VVMD27	9	0.777	0.758	0.133	0.010
VMC2h4	10	0.849	0.844	0.075	0.002
VVIb01	5	0.530	0.603	0.453	-0.047
VVS2	13	0.831	0.862	0.081	-0.016
VVMD5	11	0.849	0.844	0.074	0.002
VVMD31	8	0.718	0.672	0.187	0.026
VMC2c3	6	0.700	0.775	0.228	-0.044
ZAG62	9	0.815	0.810	0.110	0.003
ZAG79	9	0.799	0.672	0.128	0.070
ZAG83	6	0.623	0.534	0.356	0.055
Total	119	10.531	10.252		
Mean	8.500	0.752	0.714		

data directly with ours for the similarly named cultivars. In agreement with the present work, several previous reports showed that the VVS2 locus had 10 or more alleles (Sefc *et al.* 2000, Fatahi *et al.* 2003, Vouillamoz *et al.* 2006, Şelli *et al.* 2007). Karataş *et al.* (2007) found the lowest number of alleles in the VVMD5 and VVMD7 loci (10 alleles). This is consistent with our results for the same markers.

In this study, the expected heterozygosity (He) values at 9 loci (ZAG79, VVIh54, VVMD7, VVMD28, VVMD27, ZAG83, VVMD5, ZAG62 and VVMD31) were higher than the observed heterozygosity (Ho) values. Previous reports (Ibáñez *et al*. 2003, Costantini *et al*. 2005, Martinez *et al.* 2006, Karataş *et al.* 2007) also found relatively high He in some SSR loci in grapes.

Genetic relationships among Southeast Anatolian grapes: Genetic analyses performed in this study clearly separated the Mardin province from the remaining provinces (Tab. 4 and 6). The low level of gene flow estimated between Mardin and other provinces (Tab. 5) could have contributed to the distinctness of the Mardin province. Although natural selection is the most important factor creating linkage disequilibrium, higher levels of gene flow can contribute to substantial levels of disequilibrium in grape.

Synonymous and homonymous grape cultivars: Of the grape cultivars examined, one synonymous and four homonymous cultivars were found while no identical cultivars were identified. 'Tayifi' (35) and 'Reşe Drejik' ('Siyah Hatun Parmağı') (39) appear to be synonymous. These two cultivars are grown in the same location (Gercüş - Mardin) and have similar berry morphologies (Tab. 1).

Despite having different berry colors (Tab. 1), 'Abdullah' ('Apo') (21) (red berried grape) – 'Ergit' ('Asmalı') (43)

Table 4

Pairwise differentiation(F_{ST}) values

Province	Adıyaman	Diyarbakır	Gaziantep	Mardin	Siirt
Adıyaman	-				
Diyarbakır	0.00714	-			
Gaziantep	0.01190	-0.00475	-		
Mardin	0.09415***	0.09088**	0.06038*	-	
Siirt	0.00549	0.00282	-0.03427	0.04050	-
Şanlıurfa	0.01060	0.01334	0.01163	0.10242***	0.00866

* P < 0.05, ** P < 0.01, *** P < 0.001

Table 5

Gene flow values (nm) among provinces

Province	Adıyaman	Diyarbakır	Gaziantep	Mardin	Siirt
Adıyaman	-				
Diyarbakır	32.17	-			
Gaziantep	19.71	-	-		
Mardin	2.40	2.60	4.15	-	
Siirt	33.42	-	-	6.48	-
Şanlıurfa	17.88	15.22	16.85	2.16	15.89

Table 6

Genetic distances Nei (1972) among grape provinces

Province	Adıyaman	Diyarbakır	Gaziantep	Mardin	Siirt
Adıyaman	-				
Diyarbakır	0.127	-			
Gaziantep	0.180	0.154	-		
Mardin	0.395	0.382	0.338	-	
Siirt	0.191	0.205	0.145	0.294	-
Şanlıurfa	0.142	0.175	0.209	0.439	0.229

(white berried grape) and 'Tilgören' (53) (black berried grape) – 'Mazrune' ('Mazirone') (3) (white berried grape) shared the same profile at all 14 SSR loci examined in this study. Differences in berry color in otherwise genetically identical cultivars might be due to specific mutations in genes controlling berry color. In fact, Kobayashi *et al*. (2007) showed that a retrotransposon-induced mutation in *VvmybA1*, a homolog of *VlmybA1-1*, is associated with the loss of pigmentation in white berried cultivars of *V. Vinifera*. Therefore, we can not exclude the possibility that these cultivar pairs may be bud sports, since SSR markers are not powerful enough to discriminate true bud mutants from the original cultivars (Yamamato *et al.* 2003).

Genotypes with the same and/or similar names, such as 'Kuraş' (2)-'Göğ Kuraş' (5), 'Samrı' (4)-'Samrı' (10) from Adıyaman; 'Aftık' ('Hılsık Deyvani') (36)-'Siyah Aftık' ('Siyah Deyvani'/'Hılsı Kireş') (38) from Mardin; 'Mazrune' ('Mazirone') (3) from Adıyaman and 'Mazrune' ('Siverek Üzümü'/'Batık Kabarcığı') (52), were considered to be homonymous.

Apart from the two 'Samrı' cultivars, which formed a homonymous group, 'Kuraş', 'Mazrune', and 'Aftık' showed high similarity to the remaining cultivars. Cultivar 3, 'Mazrune' ('Mazirone') - or synonym 'Tilgoren' (53) - showed 96.4 % similarity to 'Kara Tümbül' (17), suggesting that 'Kara Tümbül' could be a 'Mazrune' clone. Additionally, homonym 'Kuraş' cultivars from Adıyaman, Cultivar 2 with 'Muhammediye' ('Mor üzüm') (22), and 'Göğ Kuraş' (5) with 'Kahti Göğ' (16) formed a dual group with high similarity (92.9 %). Due to similar morphology and berry color (The name "Göğ" means cloudy berry color in Turkish), it is possible that 'Gölbaşı'/Adıyaman originated from 'Göğ Kuraş' (5) and 'Kahti Göğ' (16), which are closely related cultivars. 'Siyah Aftık' ('Siyah Deyvani'/'Hılsı Kireş') (38) and 'Mazrune' ('Siverek üzümü'/'Batık Karbarcığı') (52) showed 92.9 % similarity to each other and clustered together. Furthermore, 'Aftık' ('Hılsık Deyvani') (36) showed high similarity (96.4 %) to 'Musabbık' (34), indicating that these two cultivars could be either 'Aftık' or 'Musabbık' clones.

Similar ampelographic characteristics (Tab. 1) and high genetic similarity of 'Çiloreş' (50)-'Kızlar Tahtası' (7) and 'Çiloreş-Ruhali' ('Küllahi') (49) and 'Çiloreş', 'Kızlar Tahtası' and 'Ruhali' suggest that these genotypes might

have originated from the same genetic background or one clonally derived from the other. Although, Gürsöz (1993) reported that 'Külahi' (49)-'Muhammediye' (22); 'Kunefi' (26)-'Gülgülü' (11), and 'Çiloreş' (50)-'Kızlar Tahtası' (7) were synonymous based on their ampelographic characteristics, our results from the SSR analysis provided evidence that these are distinct cultivars. Cultivar 26 ('Kunefi') was 96.4 % similar to cultivars 21 ('Abdullah' ('Apo') and its synonym 'Ergit' ('Asmalı') (43). Again, it is possible that 'Kunefi' and 'Abdullah' might have originated from the same genetic background or one clonally derived from the other. Finally, accession 24 (an unnamed cultivar) from Diyarbakır was not similar to any other cultivars while Accession 47 (another unnamed cultivar) from Siirt was 90.0 % similar to 'Gülgülü' from Adıyaman.

Homonymous grape cultivars are often found among Turkish grapes (Karataş *et al.* 2007), indicating that identically named cultivars may not be genetically the same variety. The number of synonymous detected in this study were lower than those reported previously **(**Ergül *et al.* 2006, Karataş *et al.* 2007, Şelli *et al.* 2007, Gök Tangolar *et al.* 2009). This probably reflects the higher genetic diversity values found in the cultivars analyzed here.

In conclusion, the findings reported in this paper will be useful for breeding and germplasm management of regional grape cultivars. Notably, the genetic diversity data reported here using the universially accepted set of SSR loci would allow direct comparisons to be made between the results of this study and other studies conducted in the past on other grape cultivars. Our data can also be integrated into future studies investigating the genetic diversity of grapes from other regions.

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