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Simple sequence repeat-based assessment of genetic diversity in 'Dimrit' and 'Gemre' grapevine accessions from Turkey

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Summary

It is widely believed that Turkey has played an important role in the evolution of modern day grapes due to its unique geographical location with close proximity to the regions of grape diversity. Despite this, the rich grape germplasm found in Turkey has not been sufficiently analyzed genetically. In this study, 31 grapevine accessions from 'Dimrit' (or 'Dilmit') and 'Gemre' grape groups were genetically analyzed at eight SSR (microsatellite) loci (VVS2, VVMD5, VVMD7, VVMD24, VVMD27, VVMD28, VrZAG62 and VrZAG79) and for a number of ampeolographic characteristics. These analyses identified sufficient genetic diversity between these two grape groups that, in general, clustered separately in the dendrogram constructed based on the SSR data. However, the ecogeographical distribution and genetic relationship of the genotypes did not show any significant correlation. Two 'Gemre' accessions were determined as genetically identical. In addition, one case of synonym and several cases of homonym genotypes were identified. The results reported here are important first steps towards better characterization of these grape genotypes and would aid future germplasm management and breeding efforts.

K e y w o r d s : *Vitis vinifera* L., 'Dimrit', 'Gemre', SSR, Turkey, homonym, synonym.

Introduction

Turkey has a rich grapevine (*Vitis vinifera* L.) germplasm, possibly owing to the fact that Anatolia is one of the centers of diversity for *V. vinifera* (Arroyo-Garcia *et al.* 2006). Recently, in an attempt to preserve Anatolian grape genetic resources, a grape germplasm repository called the "National Grapevine Germplasm Vineyard*"* has been established at the Institute of Viticulture in Tekirdağ, Turkey. This collection currently contains approximately 1,200 grapevine accessions collected from different locations of Turkey for over more than 30 years. Despite some ampelographic studies conducted on this collection, to date, very few studies have been carried out for genetic characterization of this grape germplasm at the molecular level (Ergül *et al*. 2002 a and b*,* 2006*,* Vouillamoz *et al*. 2006).

Among the grapevine accessions found at this repository, the accessions belonging to two grapevine groups, namely, 'Dimrit' (or 'Dilmit') and 'Gemre' are of particular importance. These two grapevine groups display relatively high genetic variation in a few economically important quality traits such as berry color, flavor, ripening time and different usage (e.g. fresh consumption and/or vine making). In addition, these grapes are among the most widely cultivated grapevines in certain localities of Turkey. Within 'Gemre' group grapes, two grape genotypes known as Pembe (Pink) and Siyah (Black) 'Gemres' are predominantly cultivated. Of these two, 'Pembe Gemres' are extensively grown in the Aegean region while Black 'Gemres' have a wider distribution and are grown in the Aegean, the Mediterranean and the Central Anatolian regions of Turkey. Currently, Pink and Black 'Gemres' together comprise 1.5 % of the total grape-grown area and 1.8 % of total grape production in Turkey (Anonymous 2007). Pink and Black 'Gemres' are consumed mostly as table grapes.

Within 'Dimrit' group of grapevines, 'Akdimrits' (White Dimrits) and 'Karadimrits' (Black Dimrits), are grown in Central Anatolia while 'Burdur Dimrits' are grown in the Mediterranean Region. 'Dimrit' group grapes comprise 2.3 % of total grape-grown area and 3.1 % of total grape production in Turkey (Anonymous 2007). The majority of 'Dimrit' grapes is consumed as traditional grape products (*e.g.* grape molasses and dried sweets made of boiled-down grape juice), raisin or table grapes and the rest are used for wine production. Moreover, a significant amount of 'Dimrit' grapes is used for the production of traditional alcoholic beverage called 'rakı'.

One of the issues often faced in studies aimed at genetic characterization of these grape groups is that 'Gemre' and 'Dimrit' grapes grown in different locations of Turkey are known by a number of different names (see below). For instance, various forms of 'Gemre' group grapes are locally known as 'Al Gemre', 'Gökçe Germe', 'Kara Germe', 'Siyah Germe' or 'Pembe Germe'. Similary, within 'Dimrit' group grapes, various grape forms are called 'Dimrit', 'Dimlit', 'Karadimrit', 'Aldimrit', 'Ak Dimrit', 'Burdur Dimriti', 'Çakır

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Dimrit', 'Çatal Dimrit', 'Siyah Dilmit' or 'Siyah Dimrit'. It is suspected that many of the 'Dimrit' and 'Gemre' accessions are in fact synonyms (the same or similar genotypes known by different names) or homonyms (genetically different genotypes known by the same names). The occurrence of homonyms and synonyms in grape germplasm has often been reported in many other studies (Dangl *et al.* 2001, Ibáñez *et al.* 2003, Martín *et al.* 2003, This *et al.* 2004, Ergül *et al.* 2006, Vouillamoz *et al.* 2006). The lack of information about the genetic relatedness of accessions within each of the 'Gemre' and 'Dimrit' group grapes is a serious factor limiting current germplasm conservation efforts and their potential utilization in grapevine breeding programs.

DNA markers provide discriminatory information and, therefore, are commonly used for germplasm characterization, cultivar and clone identification and parentage analyses. Simple Sequence Repeat (SSR or microsatellite) markers (Thomas and Scott 1993, Bowers *et al.* 1996, Sefc *et al.* 1999, Aradhya *et al.* 2003, Fatahi *et al*. 2003, Goto-Yamamoto *et al*. 2006, Regner *et al*. 2006, Vouillamoz and Grando 2006) have been used previously in *V. vinifera.*

In this study, 31 grapevine accessions belonging to 'Dimrit' and 'Gemre' groups were genetically analyzed at eight SSR loci. The allele sizes of the accessions and the genetic relationships within and between groups were determined. Synonyms and homonyms were identified and the correlation between the genetic relationship and the ecogeographical distribution of genotypes was discussed.

Material and Methods

Plant material: The grape accessions used in this study were obtained from the National Grapevine Germplasm Vineyard at the Institute of Viticulture in Tekirdağ, Turkey. The original locations and some of the ampelographic characteristics of these accessions grown and scored at the Institute's vineyard are presented in Tab. 1.

DNA isolation: DNA was extracted from young leaf tissue following the procedure described by Lefort *et al*. (1998). Concentration and purity of the DNA extracted were determined NanoDrop® ND-1000 Spectrophotometer.

SSR analysis: Eight SSR markers, namely VVS2 (Thomas and Scott 1993), VVMD5, VVMD7, VVMD24, VVMD27, VVMD28 (Bowers *et al*. 1996, 1999), VrZAG62 and VrZAG79 (Sefc *et al*. 1999) were used in this study. Six of these loci belong to the so called "core set" recommended to use for direct comparison of results from different laboratories (This *et al*. 2004). PCR amplifications were performed in a reaction volume of 10 µl reaction mixture containing 15 ng DNA, 5 pmol of each primer, 0.5 mM dNTP, 0.5 unit GoTaq DNA Polymerase (Promega, Madison, WI), including 1.5 mM $MgCl_2$. The forward primers of each pair were labeled with WellRED fluorescent dyes D2 (black), D3 (green) and D4 (blue) (Proligo, Paris, France). The 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). The analyses were repeated at least twice to ensure reproducibility of the results. Allele sizes were determined for each SSR locus using a Beckman CEQ fragment analysis software. In each run, 'Cabernet Sauvignon' and 'Pinot Noir' were included as reference cultivars.

Genetic analysis: Number of alleles (n), allele frequency, expected (H_e) and observed heterozygosity (H_o), 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 genotypes. 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 to a similarity matrix and a dendogram was constructed with UPGMA (Unweighted Pair-Group Method with Arithmetic Mean) method (Sneath and Sokal 1973), using the software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System) (version 2.0) (Rohlf 1998).

Results and Discussion

SSR analysis: The analysis of 31 'Dimrit' and 'Gemre' accessions and two reference cultivars by eight microsatellite markers generated 76 alleles sizes of which (bp) are listed in Tab. 2. The number of alleles per locus ranged from five for VVMD24 to nine for VVS2 and VVMD28 with an average allele number of 7.6. The average number of alleles found in our study is comparable to those reported in other studies on grapevines. For instance, Crespan and Milani (2001), in their analyses of 64 grapevine genotypes, used 25 markers and detected an average allele frequency of 6.58. Similarly, Dangl *et al.* (2001), in their analyses of 41 grapevine genotypes, used 11 markers and detected an average allele frequency 8.0. Other researchers have identified slightly higher average SSR allele frequencies in various grape genotypes. For instance, Ibáñez *et al.* (2003), Martín *et al.* (2003) and Vouillamoz *et al.* (2006) have reported average allele frequencies of 9.85, 11.0 and 11.5, respectively, in grapes.

The expected heterozygosity (0.7726) observed in our study is also comparable to that reported in previous studies (Sefc *et al.* 2000, Dangl *et al.* 2001, Aradhya *et al.* 2003, Fatahi *et al.* 2003, Vouillamoz *et al.* 2006). High heterozygosity is commonly found in clonally propagated and out-breeding perennial species such as grapevines (Sefc *et al.* 2000, Aradhya *et al.* 2003).

Table 1

Some ampelographic characteristics and original collection locations of 'Gemre' and 'Dimrit' group grape accessions used in this study

No.	Accession no.	Genotype name	Location (town/city/region)	Cluster form	Berry form	Berry color	Flavor	Ripening
Gemre accessions								
1	100.11	Al Gemre	Merkez/Bilecik/Central Anatolia	Long conical	Ellipsoidal	Black	*	Early October
2	204.17	Kara Gemre	Yenice/Çanakkale/Aegean	Conical	Round	Black	Sweet	Late September
3	275.59	Al Gemre	Şarköy/Tekirdağ/Thrace	Cylindrical	Ellipsoidal	Pink-black	Neutral	Late September
4	434.45	Siyah Gemre	Gördes/Manisa/Aegean	Conical	Ellipsoidal	Black	Sweet	Late October
5	496.45	Pembe Gemre (Şam)	Kırkağaç/Manisa/Aegean	Conical	Ellipsoidal	Pink	Neutral	Mid-October
6	512.42	Siyah Gemre	Akşehir/Konya/Central Anatolia	Cylindrical	Round	Black	Sweet	Mid-September
7	554.20	Pembe Gemre	Acıpayam/Denizli/Aegean	Winged conical	Round	Pink	Sweet	Late October
8	665.17	Al Gemre	Gelibolu/Çanakkale/ Aegean	Conical	Round	Black	Sweet	Late September
9	764.40	Tavşan Kanı Gemre	Mucur/ Kırşehir/Central Anatolia	Cylindrical	Long ellipsoidal	Pink	Sweet	Late September
Dimrit accessions								
10	246.23	Erkek Dilmit	Gülnar/ Mersin/Mediterranean	Conical	Round	Black	Sweet	Late September
11	252.33	Dimrit	Gülnar/ Mersin/Mediterranean	Conical	Long ellipsoidal	Black	Sweet	Late September
12	278.33	Nuri Dilmit	Gülnar/ Mersin/Mediterranean	Conical	Round	Red-black	Sweet	Early September
13	281.33	Şeker Dilmit	Gülnar/Mersin/Mediterranean	Conical	Ellipsoidal	Black	Sweet	Early September
14	285.33	Akdilmit (Ak üzüm)	Gülnar/Mersin/Mediterranean	Conical	Ellipsoidal	White	Sweet	Mid-August
15	422.42	Dilmit (Siyah Dimrit)	Hadim/Konya/Center Anatolia	Conical	Round	Black	Sweet	Late September
16	448.45	Çatal Dirmit	Gördes/Manisa/Aegean	Conical	Round	Black	Sweet	Early September
17	459.42	Siyah dimrit (Yerli)	Karaman/Konya/Central Anatolia	Cylindrical	Round	Black	Sweet	Late September
18	466.42	Erkek Dimrit	Bozkır/Konya/Central Anatolia	Cylindrical	Ovoid	Black	Neutral	Early October
19	502.20	Dimrit	Çal/Denizli/Aegean	Cylindrical	Ellipsoidal	Black	Neutral	Early August
20	508.42	Dimrit (Ak üzüm)	Akşehir/Konya/Central Anatolia	Cylindrical	Ellipsoidal	White	Neutral	Mid-September
21	513.42	Erdimrit	Akşehir/Konya/ Central Anatolia	*	Round	Black	Sweet	Early August
22	515.42	Dimrit (Kızıl üzüm)	Akşehir/Konya/Central Anatolia	Cylindrical	Round	Dark red	Sweet	Late August
23	590.20	Akdimrit (Kurutmalık)	Acıpayam/Denizli/Aegean	Cylindrical	Round	White	Neutral	Mid-August
24	599.48	Dimrit (Kayırcık)	*/ Muğla/Aegean	Winged cylindrical	Ellipsoidal	Red-black	Sweet	Mid-August
25	616.15	Akdilmit (Midri Bulut)	Center/Burdur/Mediterranean	Conical	Ellipsoidal	White	Sweet	Late September
26	438.48	İstanbul Dilmiti	Ula/Muğla/Aegean	Cylindrical	Ellipsoidal	Red	Sweet	Early August
27	653.48	Akdilmit	Ula/Muğla/Aegean	Conical	Round	Red	Neutral	Early October
28	615.15	Akdimrit	CenterBurdur/Mediterranean	Conical	Round	White	Sweet	Late September
29	657.32	Isparta Dimriti	Center/Isparta/Mediterranean	Conical	Round	Black	Sweet	Mid-September
30	758.40	Dilmit	Mucur/Kırşehir/Central Anatolia	Conical	Long ellipsoidal	White	Sweet	Early September
31	760.40	Siyah Dilmit	Merkez/Kırşehir/Central Anatolia	Winged cylindrical	Ellipsoidal	Black	Sweet	Early September

* data is not available

Table 2

Allele sizes (bp) of 31 'Dimrit' and 'Gemre' accessions at 8 loci, the reference cultivars are shown in bold (CS: 'Cabernet Sauvignon', M: 'Merlot')

No.	VVS2		VVMD5		VVMD7		VVMD24		VVMD27		VVMD28		VrZAG62		VrZAG79	
Gemre accessions																
1	141	147	225	231	242	252	211	211	181	195	243	257	188	188	258	258
2	133	143	231	233	232	248	207	207	181	185	235	243	188	202	242	250
3	137	141	231	245	238	246	207	211	181	185	235	257	186	188	250	258
4	137	143	231	235	238	246	207	211	185	185	235	235	186	188	244	244
5	141	147	225	231	242	252	215	217	181	195	243	257	188	188	258	258
6	135	141	235	239	246	246	207	207	179	185	243	277	188	204	256	256
8	137	141	231	245	238	246	207	211	181	185	235	257	186	188	250	258
7	143	149	231	239	238	250	207	207	195	195	247	257	192	204	250	258
9	135	143	235	239	238	246	205	215	181	181	243	247	188	204	250	258
Dimrit accessions																
10	135	141	235	239	246	248	205	207	179	183	257	257	188	204	256	258
11	135	141	235	239	242	248	207	217	179	185	233	257	188	200	246	246
12	143	143	235	239	238	248	207	207	179	189	233	257	188	188	242	250
13	133	141	225	239	246	246	207	211	185	195	233	257	188	192	246	246
14	135	135	235	235	242	262	207	217	185	185	255	257	200	204	242	246
15	135	141	235	239	242	250	207	217	179	185	233	257	188	200	246	250
16	131	141	235	239	242	248	207	217	179	181	247	257	194	200	250	250
17	141	141	235	239	242	248	207	217	179	185	233	257	188	200	246	250
18	135	141	235	235	246	246	207	207	179	185	255	257	188	200	242	246
19	133	133	235	239	238	242	207	207	185	189	233	243	200	204	242	250
20	135	141	235	239	246	246	207	207	181	185	235	277	188	204	248	248
21	133	141	239	239	238	242	207	217	185	185	233	247	200	204	246	246
22	141	143	225	239	238	252	207	215	181	185	247	257	188	200	242	258
23	133	141	235	239	238	248	207	207	179	185	233	257	200	204	246	246
24	131	141	237	239	238	242	207	207	185	185	233	257	188	200	242	250
25	141	141	231	235	238	248	207	207	179	185	235	235	188	200	238	250
26	131	141	237	239	238	242	207	207	185	185	233	257	188	200	242	250
27	131	141	227	235	238	248	207	207	181	185	235	257	188	202	250	258
28	143	153	225	235	238	248	207	211	179	185	243	253	200	204	248	248
29	133	149	239	239	242	246	207	207	185	189	233	243	200	204	242	250
30	131	141	235	239	248	248	205	217	179	183	243	257	188	200	250	250
31	135	141	235	235	246	248	207	207	179	185	255	255	188	200	242	246
CS	**137**	**149**	**231**	**239**	**238**	**238**	**207**	**217**	**175**	**189**	**233**	**235**	**188**	**194**	**246**	**246**
M	**137**	**149**	**225**	**235**	**238**	**246**	**207**	**211**	**189**	**191**	**227**	**233**	**194**	**194**	**258**	**258**

As far as the probability of identity (PI) is considered, the most informative loci were VVS2 and VVMD28 with nine alleles (PI: 0.100 and 0.110, respectively) while the least informative locus was VVMD24 with five alleles (PI: 0.311). The five alleles found at this locus are probably due to the low level of heterozygosity among the accessions but not due to null alleles.

The locus that generated the highest number of alleles was VVS2 also described by LÓPES *et al.* 1999, FATAHI *et al.* 2003, MARTÍN *et al.* 2003, and NÚÑEZ *et al.* 2004. In other studies, VVMD28 was reported to be the locus with the highest number of alleles (CRESPAN and MILANI 2001). Similarly, the loci, VVMD5, VVMD7, VVMD27, VVMD32, and VrZAG79, which generated eight alleles and VrZAG62, which generated seven alleles in our study were also reported to be among the most informative loci by the same researchers. Similar to that reported by VOUILLAMOZ *et al*. (2006), we identified VVMD24 as the least informative locus.

Identification of synonym and homonym accessions: SSR markers were used by many researchers to identify synonyms and homonyms of grapevine genotypes (DANGL *et al.* 2001, IBÁÑEZ *et al.* 2003, MARTÍN *et al.* 2003, THIS *et al.* 2004, VOUILLAMOZ *et al.* 2006). In 'Dimrit' and 'Gemre' group accessions analyzed here, we found only one case of 'Dimrit' synonym and three cases of 'Gemre' and four cases of 'Dimrit' homonyms (Tab. 3). Two accessions, 'Dimrit' ('Kayırcık') (599.48) collected from 'Muğla' and 'Istanbul Dilmiti' (438.48), were synonyms. These two accessions were highly similar morphologically and showed identical alleles in all loci examined (Tabs. 1, 2 and 3).

Our results also indicated that a number of accessions known by the same names, were genetically different, suggesting that these were homonyms (Tab. 3): Pembe Gemre (2 genotypes), 'Al Gemre' (100.11-275.59 and 665.17 synonyms), 'Siyah (Kara) Gemre' (3 genotypes); 'Erkek Dimrit' ('Erkek Dilmit') (2 genotypes), 'Siyah Dimrit' ('Si-

T a b l e 3

Synonyms and homonyms detected based on SSR analysis of 'Gemre' and 'Dimrit' group grapes

	Name	(No.) Accession no.						
Synonyms								
Dimrit	Dimrit (Kayırcık)-İstanbul Dilmiti	(24) 599.48	(26) 438.48					
Homonyms								
Gemre	Pembe Gemre	(5) 496.45	(7) 554.20					
	Al Gemre	(1) 100.11	(3) 275.59 or					
			(8) 665.17					
	Siyah (Kara) Gemre	(2) 204.17	(4) 434.45	(6)512.42				
Dimrit	Erkek Dimrit (Erkek Dilmit)	(10) 246.23	(18) 466.42					
	Siyah Dimrit (Siyah Dilmit)	(15) 422.42	(17) 459.42	(31) 760.40				
	Akdimrit (Akdilmit)	(27) 653.48	(28) 615.15	(25) 616.15	(23) 590.20	(14) 285.33		
	Dimrit (Dilmit)	(11) 252.33	(19) 502.20	(20) 508.42	(42) 515.42	(24) 599.48	(30) 758.40	

yah Dilmit') (3 genotypes), 'Akdimrit' ('Akdilmit') (5 genotypes), 'Dimrit' ('Dilmit') (6 genotypes).

In conclusion, ampeolographic and DNA marker studies reported here are the first ever conducted on these particular grapevine groups with relatively wide distribution in Turkey. Because we used a standard set of SSR markers with well-established reproducibility in different laboratories (This *et al.* 2004), the data reported here could be directly comparable with other studies using the same marker set. This would also allow integration of our data into future studies aiming to investigate the genetic diversity of grapes from Turkey and the surrounding regions with well-established roles in the evolution of current day grapes. Finally, it is expected that our genetic characterization of this grape germplasm, particularly the identification of homonyms and synonyms accessions etc. will help rational management of grape germplasm at the National Grapevine Germplasm Vineyard, particularly in the face of ever declining local genetic resources.

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