

Vitis 42 (4), 185–192 (2003)

Characterization of Iranian grapevine cultivars using microsatellite markers

R. FATAHI¹), A. EBADI¹), N. BASSIL^{2,3}), S. A. MEHLENBACHER²) and Z. ZAMANI¹¹) Department of Horticulture, Agricultural College, University of Tehran, Karaj, Iran²) Department of Horticulture, Oregon State University, Agricultural and Life Sciences Building, Corvallis, USA³) USDA-ARS National Clonal Germplasm Repository, Corvallis, USA

Summary

Sixty-two grapevine (*Vitis* spp.) accessions from Iran and the USA were characterized at 9 highly polymorphic microsatellite loci using fluorescent primers and a capillary electrophoresis fragment sizing system. The number of alleles observed per locus ranged from 4 to 16 and heterozygosity values ranged from 0.47 to 0.86. Genetic similarity was estimated for each pair of accessions as the proportion of shared alleles. A phenogram constructed from genetic dissimilarity values revealed three clusters, one each for table grapes, wine grapes and rootstocks. The phenogram also revealed three clonal sets (Askari, Bidane and Yaghoti) as well as some synonyms and homonyms among Iranian table grape cultivars.

Key words: *Vitis vinifera*, grape, simple sequence repeat, SSR, microsatellite.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest and most important perennial crops in the world. ALLEWELDT *et al.* (1990) estimated that there are about 14,000 cultivars, with numerous synonyms and occasional use of the same or similar names for different cultivars. The main center of diversity of *V. vinifera* is believed to stretch from Afghanistan to the south of the Caspian Sea, through the south Caucasus to the south coast of the Black Sea. Historical evidence shows winemaking by Neolithic Iranians dating to around 5400 B.C. (MCGOVERN *et al.* 1996). Important Iranian grapevine cultivars are described by TAFAZZOLI *et al.* (1993).

Trueness-to-type is necessary when planting vineyards, making wine, managing germplasm collections, choosing parents for controlled crosses, and legally protecting new cultivars (THOMAS *et al.* 1994). The large number of grapevine cultivars and clones makes correct identification and characterization challenging. Traditional ampelography, which uses morphological characters to identify cultivars, is not sufficiently reliable due to environmental influences (LAMBOY *et al.* 1998, SEFC *et al.* 1998, 1999).

Molecular markers based on the polymerase chain reaction (PCR) are being used by several labs for efficient grapevine characterization. In recent years, microsatellites, also known as simple sequence repeats (SSRs), have been the preferred type of marker for several reasons. Microsatellites

are tandemly arranged short motifs 1–6 base pairs in length, and fragment length polymorphisms are revealed by locus-specific PCR amplification. Microsatellite loci are abundant, uniformly distributed in the genome, exhibit co-dominant Mendelian inheritance and show high allelic diversity. Their reproducibility allows exchange of data among labs around the world (BOWERS *et al.* 1996, SEFC *et al.* 1999, SCOTT *et al.* 2000, DANGL *et al.* 2001, ROSSETTO *et al.* 2002). SSR markers have been developed and used for the genotyping of grapevine cultivars and clones in several labs (THOMAS and SCOTT 1993, BOTTA *et al.* 1995, BOWERS *et al.* 1996, 1999, SEFC *et al.* 1997, 1998, 1999, 2001, GRANDO and FRISINGHELLI 1998, LAMBOY *et al.* 1998, CRESPIAN *et al.* 1999, LOPES *et al.* 1999, SCOTT *et al.* 2000, REGNER *et al.* 2000, CRESPIAN and MILANI 2001, DANGL *et al.* 2001, LEFORT *et al.* 2001, FRANKS *et al.* 2002, VIGNANI *et al.* 2002).

In this study, we used 9 microsatellite markers to characterize grapevine germplasm in collections at the University of Tehran (Karaj and Varamin, Iran) and Oregon State University (OSU, Corvallis, OR, USA). The Iranian cultivars included the 5 most important groups of seedless grapes (Askari, Bidane Qermez, Bidane Sefid, Keshmeshi and Yaghoti), a few partially seeded, and a few seeded grapes. Cultivars in the OSU collection included well-known table and wine grape cultivars as well as a few rootstock cultivars.

Material and Methods

Plant material: Leaf samples were taken from vines growing in the collection of the research farm of the Horticulture Department, University of Tehran, in Karaj and Varamin, Iran. Leaves were collected from 38 of the most famous seedless and seeded table grapes that had been previously assembled from different parts of Iran. Leaves of 24 cultivars were collected from the collection at Oregon State University in Corvallis (Tab. 1), including *V. vinifera* and interspecific hybrids, and rootstocks representing *V. riparia* Scheele, *V. rupestris* Michx., and *V. champinii* Pl. Muscat Hamburg and Flame Seedless were sampled from both collections. All leaves were held at –80 °C until DNA extraction.

DNA extraction and purification: Large-scale DNA extraction was performed according to VROH BI *et al.* (1996). The resulting DNA was purified further using the method of LABRA *et al.* (2001), quantified fluorometrically, and diluted with TE buffer to 2 ng·μl⁻¹ for PCR amplification.

T a b l e 1
Grapevine cultivars characterized using microsatellite markers

Name	Collection	Use ^a	Seeds	Name	Collection	Use	Seeds
Askari	Iran	T	No	Keshmeshi Qermez Quchan	Iran	T	No
Askari Kashmar	Iran	T	No	Keshmeshi Sefid Quchan	Iran	T	No
Askari Qazvin	Iran	T	No	Keshmeshi Varamin	Iran	T	No
Askari Sefid	Iran	T	No	Khoshnav	Iran	T	Yes
Askari Seyah Shiraz	Iran	T	No	Merlot	OSU	W	Yes
Askari Shiraz	Iran	T	No	Müller Thurgau	OSU	W	Yes
Askari Sirk Shiraz	Iran	T	No	Muscat Hamburg	Iran	T,W	Yes
Askari Varamin	Iran	T	No	Muscat Hamburg	OSU	T,W	Yes
Atabaki	Iran	T	Yes	Nebbiolo	OSU	W	Yes
Beauty Seedless	OSU ^b	T	Partial	Paykani Shiraz	Iran	T	No
Bidane Khoram	Iran	T	No	Perlette	Iran	T	No
Bidane Qermez	Iran	T	No	Pinot Noir	OSU	W	Yes
Bidane Qermez Qazvin	Iran	T	No	Riparia Gloire	OSU	R	Yes
Bidane Qurvah	Iran	T	No	Rupestris St George	OSU	R	Yes
Bidane Sefid	Iran	T	No	Salt Creek	OSU	R	Yes
Bidane Sefid Qazvin	Iran	T	No	Semillon	OSU	W	Yes
Cabernet Franc	OSU	W	Yes	Seyave	Iran	T	Yes
Cabernet Sauvignon	OSU	W	Yes	Shahroudi	Iran	T	Yes
Canadice	OSU	T	No	Shiraz	OSU	W	Yes
Chardonnay	OSU	W	Yes	Sorkhak Nishabor	Iran	T	No
Cv. 12-1	Iran	T	No	Tafti Sefid	Iran	T	Yes
Dog Ridge	OSU	R	Yes	White Riesling	OSU	W	No
Dolcetto	OSU	W	Yes	Yaghoti	Iran	T	No
Fakhri Seyah	Iran	T	Partial	Yaghoti Markaz	Iran	T	No
Flame Seedless	Iran	T	No	Yaghoti Qasr	Iran	T	No
Flame Seedless	OSU	T	No	Yaghoti Qazvin	Iran	T	No
Gamay Noir	OSU	W	Yes	Yaghoti Sefid Shiraz	Iran	T	No
Gewürztraminer	OSU	W	Yes	Yaghoti Sefid Zabol	Iran	T	No
Glenora	OSU	T	Yes	Yaghoti Seyah Shiraz	Iran	T	No
Interlaken	OSU	T	No	Yaghoti Shiraz	Iran	T	No
Keshmeshi	Iran	T	No	Zinfandel	OSU	W	Yes

^a Table grape (T), Wine grape (W) or Rootstock (R).

^b Oregon State University (USA).

Amplification and allele sizing: Nine highly polymorphic loci were chosen for this study. Primers labeled with different fluorescent agents (FAM, HEX or NED) (Qiagen-Operon, Alameda, Calif. and PE Applied Biosystems, Foster City, Calif., USA) were used with a capillary electrophoresis fragment sizing system. The solution for PCR amplification (10 µl) contained 3 ng DNA, 0.04 U Biolase DNA polymerase (Bioline, Randolph, Mass., USA), 1x buffer, 0.4 mM MgCl₂, 250 µM of each dNTP and 6 pmol of each primer. The PCR program was 4 min at 95 °C, followed by 35 cycles of 92 °C for 40 s, 55-62 °C for 40 s, and 72 °C for 40 s, followed by 30 min at 72 °C in a Perkin-Elmer model 9700 thermocycler (PE Applied Biosystems, Foster City, Calif., USA). One µl of PCR product was diluted in 39 µl nanopure water as a stock and kept at -20 °C. The remainder was separated by electrophoresis in 2 % agarose gels in 1x TBE buffer, stained with ethidium bromide and visualized under UV-light, and then photographed using an imaging system (UVP Gel

Documentation System, Upland, Calif., USA) to confirm amplification. DNA fragments were analyzed on an ABI Prism 3100 Genetic Analyzer using 3100 Data Collection (1.0.1) and GeneScan (3.7) software, through a GA 3100 POP-4 (pre-formulated polymer matrix). One µl of PCR sample (diluted 200-600 times) was mixed with 11 µl of deionized formamide and 0.2 µl of red fluorescent size standard DNA (GeneScan 500 ROX), denatured for 3 min at 96 °C, and quenched in an ice bath for 5 min before injection. The size standard for detecting peaks was 500 bp, with a time setting of 44 min for data collection. Fragment size was established using the Local Southern Method.

Data analysis: Heterozygosity, allele number and frequency, and taxon-specific allele numbers were estimated for each microsatellite marker locus using the web-based MicroSat program of MINCH (1997) (<http://lotka.stanford.edu/microsat.html>). Pairwise genetic similarities were estimated using the „proportion of shared alleles” estimator (Ps) of

Bowcock *et al.* (1994), and genetic distance (D_{ps}) between pairs of accessions was calculated as $(1-P_s)$. UPGMA cluster analysis was used to construct and draw a tree from the D_{ps} matrix using MEGA2 (Kumar *et al.* 2001) software (<http://www.megasoftware.net/>).

Results and Discussion

DNA of all cultivars, clones and rootstocks was successfully amplified at all 9 loci, and fragment lengths were determined (Tab. 2). Twenty-six taxon-specific alleles were found. The number of alleles detected ranged from 4 (for locus scu14vv) to 16 (for locus VVS2) with an average of 11.4 (Tab. 3 and Fig. 1). The heterozygosity values ranged from 0.47 (for scu14vv) to 0.86 (for VVS2) with an average of 0.76 for the 9 loci (Tab. 3). Since the cultivars used in this study are not a natural population, nor are they derived from one, pairwise genetic distances were estimated based on the “proportion of shared alleles”, a measure that is suited to use with highly variable loci and unnatural populations (DANGL *et al.* 2001). Among the studied cultivars, dissimilarity based on proportion of shared alleles ranged from zero to one.

As expected, the Muscat Hamburg collected in Tehran and OSU showed identical profiles, as did Flame Seedless in the two collections. Khoshnav and Seyave, which are important for non-irrigated plantings in western Iran, also showed identical profiles. Atabaki, which appears as a unique cultivar in the phenogram, is partially seeded and has large round berries but shows a high frequency of shot berries when pollination conditions are not suitable. The partially seeded Fakhri Seyah and large-berried, seeded Tafti Sefid also appear as unique cultivars in the phenogram. The other cultivars appear as groups of identical individuals and closely related cultivars in the dendrogram (Fig. 2) and are discussed below.

Bidane Sefid and Bidane Qermez group: This group includes 7 accessions that are morphologically indistinguishable except for berry color. The berries of Bidane Sefid, Bidane Khoram, Bidane Qurvah, Bidane Sefid Qazvin

and Keshmeshi Sefid Quchan are white, while those of Bidane Qermez and Bidane Qermez Qazvin are red. The 5 white-fruited cultivars seem to be synonyms, as do the two red-fruited cultivars. A somatic mutation appears to be responsible for the difference in berry color. Difference in berry color is a commonly observed type of somatic mutation (MÜLLER-STOLL 1950, BREIDER 1953). Several other researchers have been unable to distinguish among berry color mutants using microsatellite markers. BOWERS *et al.* (1996) could not distinguish among Pinot noir, Pinot gris and Pinot blanc. CRESPIAN and MILANI (2001) identified several Muscat accessions that exhibited variation in berry color but could not be distinguished using microsatellite markers. In a study of Portuguese wine grape cultivars, LOPES *et al.* (1999) were unable to distinguish the white-berried Verdelho dos Acores and Verdelho da Madeira from the red-berried Verdelho roxo. SEFC *et al.* (1998) reported 4 additional cultivar pairs that could not be distinguished using microsatellite markers: Silvaner rot and Silvaner grün, Portugieser blau and Portugieser grün, Gutedel rot and Gutedel weiss, and Rheinriesling and Riesling rot.

Yaghoti group: Yaghoti is very early ripening, coming to market about one month earlier than other Iranian cultivars. In this group, Yaghoti Markaz (red berry), Yaghoti Seyah Shiraz (dark-red berry), Yaghoti Sefid Zabol (white berry), Yaghoti (red berry), and Yaghoti Qazvin (red berry) showed identical profiles. Two other cultivars showed similar profiles but each differed from the 5 listed above at one locus: Sorkhak Nishabor at *ssrVrZAG21* and Yaghoti Qasr at *VVS2*. Thus these two cultivars are different from Yaghoti and from each other. However, Yaghoti Shiraz and Yaghoti Sefid Shiraz, that were thought to be members of the same group, showed different SSR profiles and were placed far from the members of this group in the dendrogram.

Askari group: Six cultivars [Askari (oval berry with sharp tip), Askari Varamin (oval berry), Askari Sefid (round berry), Askari Qazvin (round berry), Askari Kashmar (oval berry), and Paykani Shiraz (finger-shaped berries)] have identical DNA profiles. Several of the cultivars from Shiraz, one of the most important areas for grapevine plantings in Iran, show unique genotypes. Askari Sirk Shiraz with round ber-

Table 2

SSR loci used for grapevine identification

Locus	Label	Size (bp)	No. of alleles	Heterozygosity	Annealing Temp. (°C)	Reference
VVS2	NED (yellow)	120-157	16	0.86	55	THOMAS and SCOTT 1993
VVMD5	HEX (green)	221-264	13	0.82	56	BOWERS <i>et al.</i> 1996
VVMD32	FAM (blue)	233-270	11	0.74	60	BOWERS <i>et al.</i> 1999
VVMD36	HEX (green)	236-472	15	0.85	62	BOWERS <i>et al.</i> 1999
<i>ssrVrZAG21</i>	NED (yellow)	189-214	11	0.78	60	SEFC <i>et al.</i> 1999
<i>ssrVrZAG47</i>	FAM (blue)	151-189	14	0.83	62	SEFC <i>et al.</i> 1999
<i>ssrVrZAG79</i>	NED (yellow)	235-264	13	0.82	58	SEFC <i>et al.</i> 1999
scu10vv	FAM (blue)	196-213	6	0.675	58	SCOTT <i>et al.</i> 2000
scu14vv	HEX (green)	165-183	4	0.47	56	SCOTT <i>et al.</i> 2000

Table 3

Genetic profile of 62 grapevine accessions at 9 microsatellite loci (allele sizes in base pairs)

Name	VVS2	VVMDS	VVMDS32	VVMDS36	Loci			SSR10vv	SSR14vv
					ssrVrZAG21	ssrVrZAG47	ssrVrZAG79		
Askari	138:142	231:235	248:270	246:251	201:205	155:170	248:256	204	179
Askari Kashmar	138:142	231:235	248:270	246:251	201:205	155:170	248:256	204	179
Askari Qazvin	138:142	231:235	248:270	246:251	201:205	155:170	248:256	204	179
Askari Sefid	138:142	231:235	248:270	246:251	201:205	155:170	248:256	204	179
Askari Seyah Shiraz	138:140	233:235	248:254	251:263	199:201	161:170	248:256	204	165:179
Askari Shiraz	138:144	221:235	248:270	251:263	205	170	246:256	201:204	165:179
Askari Sirk Shiraz	138:142	231:235	248:250	246:251	201:205	155:170	248:256	204	179
Askari Yaramin	138:142	231:235	248:270	246:251	201:205	155:170	248:256	204	179
Atabaki	142:153	231:235	248:260	239:263	189:201	155:170	244:256	204	179
Beauty Seedless	120:149	231:235	248	263:291	199:201	157	244:262	204	179
Bidane Khoram	142:149	231	248	246:263	189:201	157:170	244:256	198:204	179
Bidane Qermez	142:149	231	248	246:263	189:201	157:170	244:256	198:204	179
Bidane Qermez Qazvin	142:149	231	248	246:263	189:201	157:170	244:256	198:204	179
Bidane Qurvah	142:149	231	248	246:263	189:201	157:170	244:256	198:204	179
Bidane Sefid	142:149	231	248	246:263	189:201	157:170	244:256	198:204	179
Bidane Sefid Qazvin	142:149	231	248	246:263	189:201	157:170	244:256	198:204	179
Cabernet Franc	136:140	223:237	238:256	249	189:199	157:165	244:256	198:210	165:179
Cabernet Sauvignon	136:149	228:237	238	249:259	199:205	151:165	244	201:210	165
Canadice	129:149	235	248:270	259:263	199:205	161	244:262	196	165:179
Chardonnay	134:140	231:235	238:270	249:271	199:205	157:165	241:243	204:213	165:179
Cv. 12-1	138:140	233:235	248:254	251:263	199:201	161:170	248:256	204	165:179
Dog Ridge	129:138	260	235:267	236	193:214	167:189	237:253	201	183
Dolcetto	136:140	231:243	260:270	247:259	205	155:170	246:248	198:204	165:179
Fakhri Seyah	138:140	235:237	254:270	263:273	199:205	161:169	246:248	204	179
Flame Seedless (IR)	129:149	231:233	248:270	249:263	189:199	157:161	244:248	204:210	179
Flame Seedless (US)	129:149	231:233	248:270	249:263	189:199	157:161	244:248	204:210	179
Gamay Noir	129:134	231:235	238:270	249:259	199:205	157:165	241:243	201:210	165:179
Gewürztraminer	149	228:235	238:270	249:259	199:205	165	243:248	201:204	165:179
Glenora	121:149	231:235	248	246:291	189:205	170	248:262	204	179
Interflaken	129:149	231:233	248:270	246:271	201:212	157	244:253	204:210	179
Keshmeshi	138:142	231:233	248:270	246:263	189:205	161:170	248:256	198:204	165:179
Keshmeshi Qermez Quchan	138:149	231:243	248:270	246:283	201:205	155:170	255:256	204	165:179

Tab. 3, continued

Name	Loci									
	VVS2	VVMD5	VVMD32	VVMD36	ssrVrZAG21	ssrVrZAG47	ssrVrZAG79	SCU10vv	SCU14vv	
Keshmehi Sefid Quchan	142:149	231	248	246:263	189:201	157:170	244:256	198:204	179	
Keshmehi Varamin	132:138	231:243	248:254	251:259	189:205	155:170	244:256	204	179	
Khoshnav	129:140	226:243	248:254	251:273	189:201	161:169	244:248	204	173:179	
Merlot	136:149	223:233	238	249	199	165:167	256	198:213	179	
Müller Thurgau	140:149	223:225	250	249:259	201	157	241:243	198:204	165:179	
Muscat Hamburg (IR)	131:147	228:235	270	249:291	189:205	155:161	237:253	204:210	179	
Muscat Hamburg (US)	131:147	228:235	270	249:291	189:205	155:161	237:253	204:210	179	
Nebbiolo	153	228:233	238:260	259:472	189	161:165	241:248	204:210	165	
Paykani Shiraz	138:142	231:235	248:270	246:251	201:205	155:170	248:256	204	179	
Perlette	129:142	231:233	248:270	263:271	189:212	157	244:253	204:210	179	
Pinot Noir	134:149	225:235	238:270	249	199:205	161:165	237:243	201:213	165:179	
Riparia Gloire	138:142	262	233:235	236:248	203:208	183:187	253:256	201:204	183	
Rupestris St. George	134	233:264	233:235	236	203:206	181:183	256:260	204	183	
Salt Creek	125:129	260:264	235	236	203	162	255:264	201:204	183	
Semillon	129	233:235	238:270	259:472	199:205	151:161	244	198:204	179	
Seyave	129:140	226:243	248:254	251:273	189:201	161:169	244:248	204	173:179	
Shahroudi	136:149	221:231	248:270	239:246	189:205	157:161	248:256	198:204	165	
Shiraz	129	223:228	238:270	249:291	189:205	165:167	243:248	201:204	165:179	
Sorkhak Nishabor	140:142	231:237	248:270	246:263	199:205	157:161	244:248	198	179	
Tafti Sefid	134:149	237	248:260	263:271	189	159:170	246:255	198:204	179	
White Riesling	140:149	223:231	250:270	249:259	201:205	157:165	241:243	201:204	165:179	
Yaghoti	140:142	231:237	248:270	246:263	189:205	157:161	244:248	198	179	
Yaghoti Markaz	140:142	231:237	248:270	246:263	189:205	157:161	244:248	198	179	
Yaghoti Qasr	157	231:237	248:270	246:263	189:205	157:161	244:248	198	179	
Yaghoti Qazvin	140:142	231:237	248:270	246:263	189:205	157:161	244:248	198	179	
Yaghoti Sefid Shiraz	140	221:237	248:270	259:263	201:205	161:169	248	198:204	165:179	
Yaghoti Sefid Zabol	140:142	231:237	248:270	246:263	189:205	157:161	244:248	198	179	
Yaghoti Seyah Shiraz	140:142	231:237	248:270	246:263	189:205	157:161	244:248	198	179	
Yaghoti Shiraz	144:153	221:231	248:270	263:265	201:205	170	246:248	201:204	165:179	
Zinfandel	129:140	223:233	254:262	249	200:206	157	235:256	201	179	

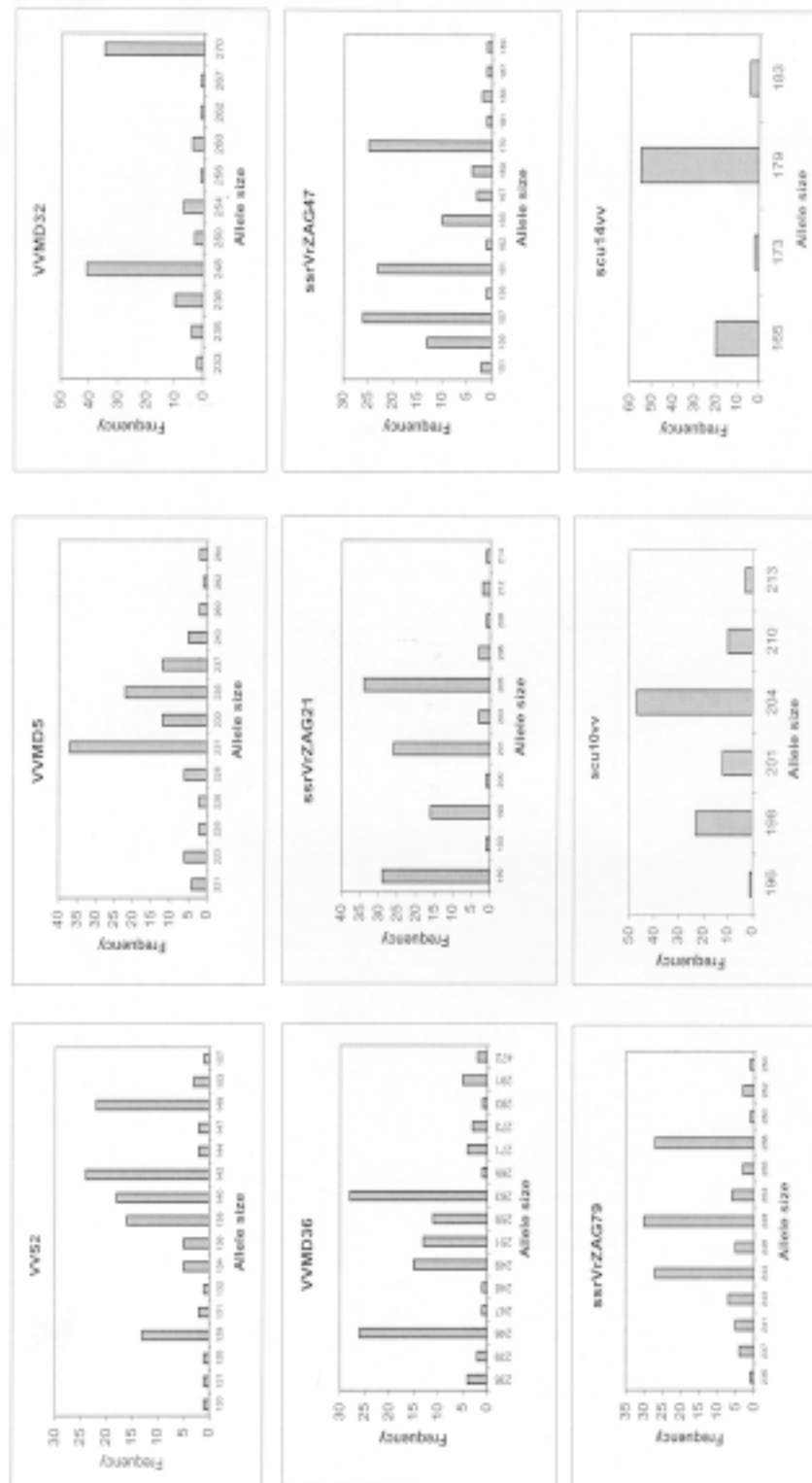


Fig. 1: Allele sizes (in base pairs) at nine microsatellite loci in 62 grapevine accessions.

ries is similar to the 6 other cultivars in the Askari group. However, Askari Shiraz and Askari Seyah Shiraz were not placed in the Askari group. The profile of unknown cultivar 12-1 was identical to that of Askari Seyah Shiraz. Both have round, red berries and their different leaf shape distinguishes them from other members of the Askari group (0.39 dissimilarity).

Keshmeshi group: The nature of the 4 Keshmeshi cultivars, all used for raisins, is very unclear. All were ex-

pected to group closely to the Bidane group, but only Keshmeshi Sefid Quchan showed an identical profile with the 6 cultivars in the Bidane group. The other three showed an average dissimilarity of 0.41, and appeared in different branches of the tree (Fig. 2). Thus, the Keshmeshi cultivars appear to be homonyms; names with the same or similar sound were given to different genotypes. Shahroudi, a very late-ripening and extremely seeded cultivar with big pinkish berries, shares one allele at each locus with Keshmeshi (seed-

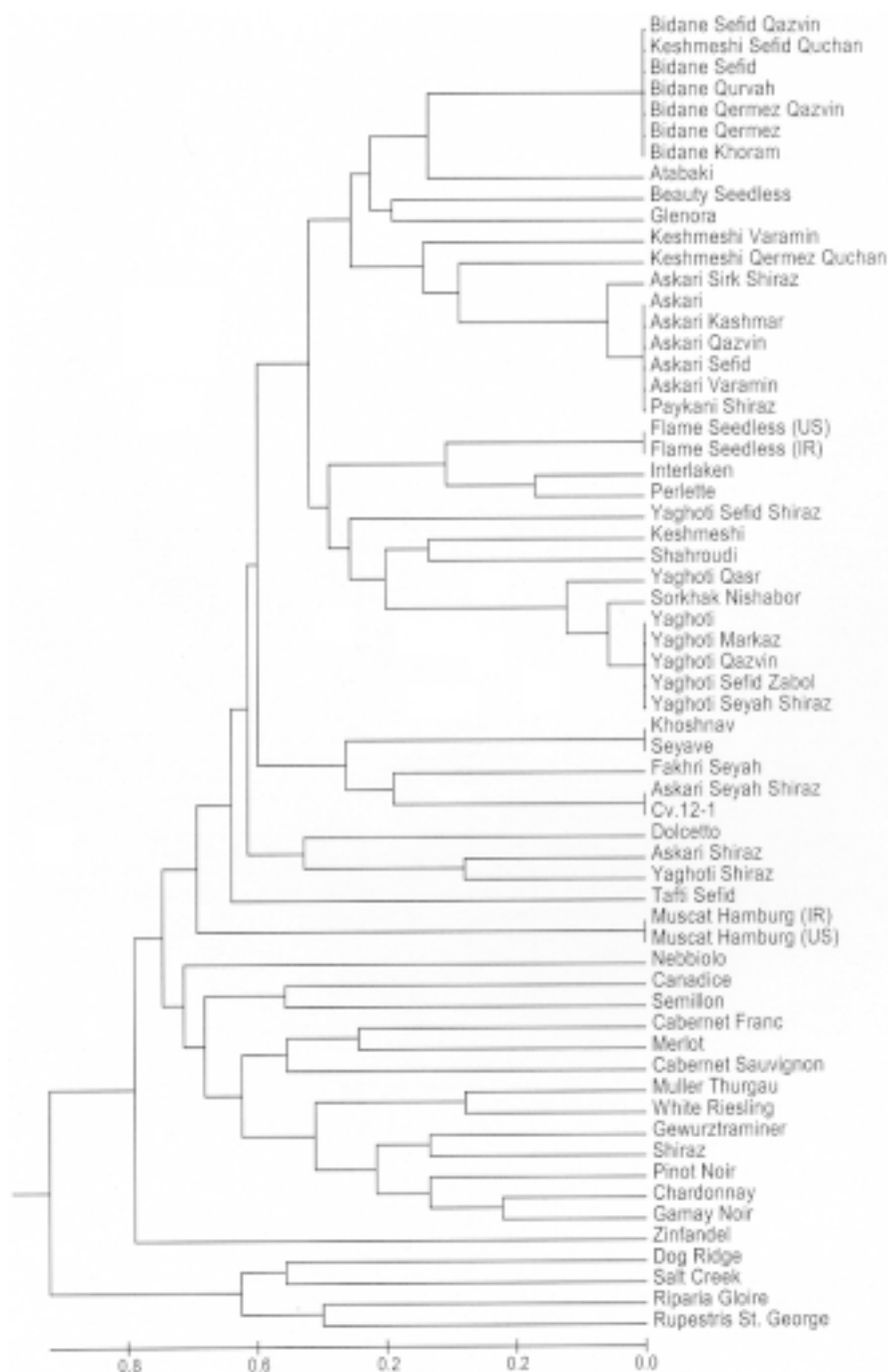


Fig. 2. Dendrogram of 62 grapevine accessions. Genetic dissimilarity was calculated as (1- the proportion of shared alleles).

less, mid-season ripening with small white berries). It appears that these two cultivars have one parent in common or have a parent-offspring relationship.

Wine grape group: All wine grapes were grouped loosely in a single sub-cluster except Dolcetto, which was on the border between the table and wine grapes, and Zinfandel, which was placed between the wine grapes and rootstocks. Canadice was an exception in that this hybrid slip-skin seedless table grape grouped with the wine grapes.

North American seedless table grapes: Glenora (Ontario Russian Seedless) and Beauty Seedless (Scolokertek Kiralynoje Black Kishmish) with only 0.44 dissimilarity grouped together. According to DANGL *et al.* (2001), Russian Seedless and Black Kishmish are synonyms,

and thus Glenora and Beauty Seedless have one parent in common. Another hybrid group was Perlette, Interlaken, and Flame Seedless, which share one allele at each locus. The first two are seedlings of Sultanina, while Sultanina appears twice in the pedigree of Flame Seedless. Comparison of allele sizes with those reported for Sultanina at 7 loci indicates that the shared alleles are derived from Sultanina (SEFC *et al.* 1998, CRESPIAN *et al.* 2001). No information has been published for Sultanina alleles at the scu10vv and scu14vv loci.

Rootstock group: The 4 rootstocks Rupestris St. George, Riparia Gloire, *V. champinii* Salt Creek and *V. champinii* Dog Ridge formed an independent cluster. This was surprising because these rootstock selections represent three different North American species.

Conclusions

SSR marker data with genetic distances calculated from the proportion of shared alleles and UPGMA clustering seems to be a very efficient method for characterization of cultivars of grapevine and other clonally propagated crops. The 62 accessions used in this study included table grapes, wine grapes, and rootstocks. As expected, the duplicate accessions of Muscat Hamburg and Flame Seedless showed identical profiles. Among the Iranian table grape cultivars, 6 additional groups with identical profiles were seen. The three largest sets of Iranian cultivars were Askari, Bidane and Yaghoti. The SSR markers in this study could not differentiate among clones of a cultivar, including variations in berry color or shape that are likely the result of somatic mutation (FRANKS *et al.* 2001). Additional molecular markers may be able to distinguish among these clones and sports, as reported by REGNER *et al.* (2000) for White Riesling clones. Keshmesh, a name that means raisin, is a very common name of the grapes used for making raisins in Iran. In this case, similar names imply identical use but do not necessarily indicate genetic similarity of cultivars. The Keshmeshi grapes in this study show dissimilarities ranging from 0.39 to 0.50 and are thus homonyms.

References

- ALLEWELDT, G.; SPIEGEL-ROY, P.; REISCH, B.; 1990: Grapes (*Vitis*). In: J. N. MOORE and J. R. BALLINGTON (Eds.): Genetic Resources of Temperate Fruit and Nut Crops. Acta Hort. **290**, 291-337.
- BOTTA, R.; SCOTT, N. S.; EYNARD, I.; THOMAS, M. R.; 1995: Evaluation of microsatellite sequence-tagged site markers for characterizing *Vitis vinifera* cultivars. *Vitis* **34**, 99-102.
- BOWERS, J. E.; DANGL, G. S.; VIGNANI, R.; MEREDITH, C. P.; 1996: Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome* **39**, 628-633.
- BOWERS, J. E.; DANGL, G. S.; VIGNANI, R.; MEREDITH, C. P.; 1999: Development and characterization of additional microsatellite DNA markers for grape. *Am. J. Enol. Vitic.* **50**, 243-246.
- BOWCOCK, A. M.; RUIZ-LINARES, A.; TOMFOHRDE, J.; MINCH, E.; KIDD, J. R.; CAVALLI-SFORZA, L. L.; 1994: High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* **368**, 455-457.
- BREIDER, H.; 1953: The somatic mutations of grape and their relations with the structure of branches (in French). *Prog. Agric. Vitic.* **139**, 43-47, 70-73.
- CRESPIAN, M.; BOTTA, R.; MILANI, N.; 1999: Molecular characterization of twenty seeded and seedless table grape cultivars (*Vitis vinifera* L.). *Vitis* **38**, 87-92.
- CRESPIAN, M.; MILANI, N.; 2001: The Muscats: A molecular analysis of synonyms, homonyms and genetic relationships within a large family of grapevine cultivars. *Vitis* **40**, 23-30.
- DANGL, G. S.; MENDUM, M. L.; PRINS, B. H.; WALKER, M. A.; MEREDITH, C. P.; SIMON, C. J.; 2001: Simple sequence repeat analysis of a clonally propagated species: A tool for managing a grape germplasm collection. *Genome* **44**, 432-438.
- FRANKS, T.; BOTTA, R.; THOMAS, M. R.; 2002: Chimerism in grapevine: Implications for cultivar identity, ancestry and genetic improvement. *Theor. Appl. Genet.* **104**, 192-199.
- GRANDO, M. S.; FRISINGHELLI, C.; 1998: Grape microsatellite markers: Sizing of DNA alleles and genotype analysis of some grapevine cultivars. *Vitis* **37**, 79-82.
- KUMAR, S.; TAMURA, K.; JAKOBSEN, I. B.; NEI, M.; 2001: MEGA2: Molecular evolutionary genetics analysis software. *Bioinformatics* **17**, 1244-1245.
- LABRA, M.; CARREÑO-SANCHEZ, E.; BARDINI, M.; BASSO, B.; SALA, F.; SCIENZA, A.; 2001: Extraction and purification of DNA from grapevine leaves. *Vitis* **40**, 101-102.
- LAMBOY, W. F.; ALPHA, C. G.; 1998: Using simple sequence repeats (SSRs) for DNA fingerprinting germplasm accessions of grape (*Vitis* L.) species. *J. Am. Soc. Hortic. Sci.* **123**, 182-188.
- LEFORT, F.; ROUBELAKIS-ANGELAKIS, K. K. A.; 2001: Genetic comparison of Greek cultivars of *Vitis vinifera* by nuclear microsatellite profiling. *Am. J. Enol. Vitic.* **52**, 101-108.
- LOPES, M. S.; SEFC, K. M.; ERAS DIAS, E.; STEINKELLNER, H.; LAIMER DA CÂMARA MACHADO, M.; DA CÂMARA MACHADO, A.; 1999: The use of microsatellites for germplasm management in a Portuguese grapevine collection. *Theor. Appl. Genet.* **99**, 733-739.
- MCGOVERN, P. E.; GLUSKER, D. L.; EXNER, L. J.; VOIGT, M. M.; 1996: Neolithic resinated wine. *Nature* **381**, 480-481.
- MINCH, E.; 1997: MicroSat, Version 1.5b. Stanford University Medical Center, Palo Alto, Calif. (<http://hpgl.stanford.edu/projects/microsat/>)
- MÜLLER-STOLL, W. R.; 1950: Mutative Färbungsänderungen bei Weintrauben. *Züchter* **20**, 288-291.
- REGNER, F.; WIEDECK, E.; STADLBAUER, A.; 2000: Differentiation and identification of White Riesling clones by genetic markers. *Vitis* **39**, 103-107.
- ROSSETTO, M.; MCNALLY, J.; HENRY, R. J.; 2002: Evaluating the potential of SSR flanking regions for examining taxonomic relationships in the Vitaceae. *Theor. Appl. Genet.* **104**, 61-66.
- SCOTT, K. D.; EGGLE, P.; SEATON, G.; ROSSETTO, M.; 2000: Analysis of SSRs derived from grape ESTs. *Theor. Appl. Genet.* **100**, 723-726.
- SEFC, K. M.; STEINKELLNER, H.; WAGNER, H. W.; GLÖSSL, J.; REGNER, F.; 1997: Application of microsatellite markers to parentage studies in grapevine. *Vitis* **36**, 179-183.
- SEFC, K. M.; REGNER, F.; GLÖSSL, J.; STEINKELLNER, H.; 1998: Genotyping of grapevine and rootstock cultivars using microsatellite markers. *Vitis* **37**, 15-20.
- SEFC, K. M.; REGNER, F.; TURETSCHKE, E.; GLÖSSL, J.; STEINKELLNER, H.; 1999: Identification of microsatellite sequences in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. *Genome* **42**, 367-373.
- SEFC, K. M.; LEFORT, F.; GRANDO, M. S.; SCOTT, K. D.; STEINKELLNER, H.; THOMAS, M. R.; 2001: Microsatellite markers for grapevine: A state of the art. In: K. A. ROUBELAKIS-ANGELAKIS (Ed.): Molecular Biology and Biotechnology of grapevine. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- TAFAZZOLI, A.; HEKMATI, J.; FIROUZEH, M.; 1993: Grape (in Farsi), 2nd Ed. Shiraz University Press, Shiraz, Iran.
- THOMAS, M. R.; SCOTT, N. S.; 1993: Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). *Theor. Appl. Genet.* **86**, 985-990.
- THOMAS, M. R.; CAIN, P.; SCOTT, N. S.; 1994: DNA typing of grapevines: A universal methodology and database for describing cultivars and evaluating genetic relatedness. *Plant Mol. Biol.* **25**, 939-949.
- VIGNANI, R.; SCALI, M.; MASI, E.; CRESTI, M.; 2002: Genomic variability in *Vitis vinifera* L. „Sangiovese” assessed by microsatellite and non-radioactive AFLP test. *Electr. J. Biotechnol.* **5**, 1-11.
- VROH, B. I.; HRAVENGT, L.; CHANDELIER, A.; MERGEAI, G.; DU JARDIN, P.; 1996: Improved RAPD amplification of recalcitrant plant DNA by the use of activated charcoal during DNA extraction. *Plant Breed.* **115**, 205-206.

Received November 19, 2002