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Full Length Research Paper

Molecular and ampelographic characterization of some grape hybrids (*Vitis vinifera* L.)

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23 hybrids were selected as potential cultivar candidates due to their superior characteristics and were created at the Atatürk Horticultural Central Research Institute (AHCRI) or the Tekirdağ Viticulture Research Institute (TVRI). In this study, the ampelographic and molecular characteristics of 23 grapevine hybrids created in two different breeding studies were determined. Ampelographic data were collected during two vegetation periods in Yalova, Turkey. Total of 68 ampelographic characteristics were identified for 23 hybrids. Polymerase chain reaction (PCR) - based microsatellite technique was used to analyse 23 hybrids and 2 standard varieties. The genetic relationships were defined with 20 simple sequence repeat (SSR) markers, and genetic similarity indices, principal component analysis graphs and phylogenetic relationship dendrograms were produced depending on the hybrid and SSR marker size of the allele which ranged from 88 to 294 bp. Additionally, ampelographic and molecular scores were evaluated together. After evaluation of molecular and ampelographic data together, similarity ratio was increased and ampelographic characterisation was dominated by molecular characterization. It may be related using more ampelographic characters in the study. Variety candidates which were obtained from different Institutes were characterized and taken under protection with this study. The information in the obtained database can be used for cultivar identification, parentage analysis, and legal protection. Also, some ampelographic characterization results will be used in determining the priority of registration.

Key words: Ampelography, characterization, hybrids, simple sequence repeat (SSR), *Vitis vinifera* L.

INTRODUCTION

Grapevines (*Vitis vinifera* L.) are one of the oldest and most important perennial crops in the world. The wide biodiversity of grapevine germplasm provides invaluable resources to breeders. However, due to common vegetative propagation methods, the long history of viticulture and the reliance on ampelography in taxonomic studies, contradictions in the definitions of genotypes are observed (Thomas et al., 1993). Although Alleweldt et al. (1990) estimated that 14.000 grape cultivars exist, a single cultivar may possess numerous synonyms, and the same name or similar names are often used for genetically different cultivars.

Grapevine diversity originated in Turkey, and many local grapevine cultivars possess desirable characteristics. According to Oraman and Ağaoğlu (1969), Turkey has a history of viticulture dating back to 3500 B.D. Moreover, viticulture and wine production were established in eastern and south-eastern Anatolia (Çelik et al., 2000).

Grape breeding programs are conducted in many countries; however, these programs vary in scope and size. Some breeding programs are focused on the

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production of rootstocks, wine, dessert grapes and raisins (Janick and Moore, 1996). In Turkey, breeding studies began in the 1970s and are currently in progress at various institutions. As a result, 12 new table grape cultivars have been registered and several cultivars from these studies have been selected (Uslu and Samancı, 1998; Özer et al., 2005).

The ampelography is the concrete study of the ampelographical characterisation of the grapes (Ampelo = grape, graphy = description). The history of the ampelography dates back to Plinius and Columella in the 1st century. Ampelographic studies have been conducted since the 17th century, and the characteristics of many grapes have been determined by different researchers in various countries (Martinez and Perez, 2000; Russo et al., 2004; Cangi et al., 2006). Previously, the determination of grape characteristics was based on the descriptors for grapevine (*Vitis* spp.) prepared by OIV-IBPGR-UPOV charts in 1983. However, the latest list was developed in 1997 and 2001 (GENRES-081 1983, 1997; OIV 2001).

Microsatellite markers offer an objective means of identifying cultivars. Microsatellite markers have also been utilised to verify or clarify the genotype and parentage of particular grape lines (Bowers and Meredith, 1997). Microsatellites may also have applications in the characterisation of new and unique cultivars for the purpose of patents and plant variety rights. To overcome the existing confusion in grapevine nomenclature, simple sequence repeat (SSR) markers for the genetic analysis of grapevine cultivars have been evaluated (Thomas and Scott, 1993; Bowers et al., 1996; Sefc et al., 1999; Lefort et al., 2002). One of the major applications of SSR markers in grapevines is the identification and discrimination of cultivars for the management of cultivar collections and the control of plant material sales (Sefc et al., 2000).

Compared to DNA fingerprinting techniques, ampelographic all traits are less reliable and are inefficient for the precise discrimination of closely related genotypes and the analysis of their genetic similarities. Alternatively, ampelographic all traits are useful for preliminary, rapid, simple and inexpensive varietal identification and can be used as a general approach for assessing genetic diversity among phenotypically distinguishable cultivars. However, the analysis of ampelographical traits is cost inefficient and time consuming (Martinez et al., 2003).

Nowadays using ampelographic and molecular determination simultaneously is getting more common by some researchers (Söylemezoğlu et al., 2001; Roldán-Ruiz et al., 2001; Martinez et al., 2003). 44 grapevine cultivars were characterized simultaneously with ampelographic and molecular markers by Sabır et al. (2009). 41 ampelographic data were obtained during two vegetation periods. 60 ISSR markers were also employed to characterize the genotypes at the DNA level.

The objectives of the present study were to characterise grapevine cultivar candidates obtained from two different institutions by employing ampelographic and simple sequence repeat-polymerase chain reaction (SSR-PCR) techniques and to determine the discriminative powers of SSR primers across genotypes. Moreover, dendrogram were obtained from the two different approaches, and the results were compared. As a result, several characteristics of the candidates were determined prior to registration.

MATERIALS AND METHODS

23 hybrid grapevines were analysed to determine their ampelographic and molecular characteristics (Table 1). 12 of the 23 hybrids were created at the Atatürk Horticultural Central Research Institution (AHCRI), and 11 hybrids were breeded at the Tekirdağ Viticulture Research Institution (TVRI). The vines were approximately eight years old and were grown under identical conditions. Hybrids were grafted Kober 5 BB rootstock modified double T training system and drip irrigation also was used for all hybrids. Alphonse Lavallée and Italia cultivars were used as standard varieties during the molecular characterisation.

Ampelographic evaluation

Ampelographic characterisation of the candidates was conducted using the descriptions in the Descriptors for Grapevine (*Vitis* spp.) (GENRES 081 1997) and the Office International de la Vigne et du Vin (OIV) Descriptor List for Grape Varieties and Vitis species (OIV, 2001). The latter was used preferentially, and the former was utilised as a supplement. Highly discriminating characteristics were selected according to the recommendation of the OIV descriptor list for grape varieties and *Vitis* species. Descriptors used in this study and their OIV-IPGRI codes are presented in Table 2. In total, 68 different descriptors were used.

Ampelographic observations were made during two consecutive vegetation periods in 2008 and 2009. The characteristics of the vines were defined and measured according to OIV descriptors. The shoot tips were investigated when they were approximately 10 to 30 cm in height, and the first four distal leaves of young leaves were evaluated.

Mature leaf descriptions were obtained between berry set and beginning of berry maturity and were conducted on leaves above the cluster within the middle of the shoot. The clusters were measured at maturity, and berry characteristics were obtained from ripe berries located in the middle of the bunch.

On average, ten canes per variety were analysed after leaf fall. The mean values obtained over two years were transformed to numerical scales according to international descriptors. The resulting raw data were analysed in NTSYSpc 2.0 software (Rohlf 2000) using a distance matrix. The clustering dendrogram was based on the unweighted pair group of the arithmetic mean (UPGMA) (Rohlf, 2000). A principal component analysis (PCA) graph was also constructed.

DNA extraction and molecular analysis

Fresh and young leaves were harvested for DNA extraction and SSR analysis from the AHCRI vineyard and ground into a fine powder with liquid nitrogen using a sterile mortar and pestle. Genomic DNA was extracted from young leaves with a Qiagen DNeasy plant mini kit according to the manufacturer's protocol (DNeasy Plant Handbook). The quality and concentration of DNA were verified on agarose gels and were measured by spectrophotometry. A dilution test was conducted to determine the 30 ng/µl DNA for PCR amplification.

Table 1. The location, parents, fruit colour and seed types of 23 candidate hybrid grapevine.

Cultivar candidates	Parents	Colour	Seed	Institute
7/1	İskenderiye Misketi x Beyaz Şam	Yellow	Seed	AHCRI
5/2	Siyah Gemre x Cardinal	Yellow	Seed	AHCRI
70/1	Hafızali x Cardinal	Yellow	Seed	AHCRI
95/3 (İsmetbey)*	Siyah Gemre x Royal	Black	Seed	AHCRI
91/3 (Pembe77)*	Alphonse Lavallée x Muscat Reine des Vignes	Dark Pink	Seed	AHCRI
43/1	Beyaz Şam x Müşküle	Yellow	Seed	AHCRI
ÇH1 (Atak77)*	Beyaz Çavuş X Hamburg Misketi	Yellow	Seed	AHCRI
130/1	63 (Beyrut Hurması x Perlette) x Siyah Çekirdeksiz	Yellow	Seedless	AHCRI
53/1	Müşküle x Beyaz Şam	Yellow	Seed	AHCRI
83/1	Pembe Gemre x Cardinal	Red	Seed	AHCRI
85/1	Beyaz Çavuş x Perle de Csaba	Yellow	Seed	AHCRI
86/1	Hafızali x Muscat Reine des Vignes	Yellow	Seed	AHCRI
7/S-176*	Italia x Superior Seedless	Yellow	Seed	TVRI
26/D-3*	Kırmızı Şam X Barış	Pink	Seedless	TVRI
16/A-101	Uşuvi x Sultani Çekirdeksiz	Yellow	Seedless	TVRI
15 /A- 61*	İskenderiye Misketi x Sultani Çekirdeksiz	Yellow	Seedless	TVRI
29/C-52*	Queen x Beauty Seedless	Yellow	Seed	TVRI
15/B-56	İskenderiye Misketi x Perlette	Yellow	Seedless	TVRI
BX2-149*	Italia x Favli	Black	Seed	TVRI
FX1-1	Amasya Beyazı x 28/259	Yellow	Seed	TVRI
FX1-10	Amasya Beyazı x 28/259	Yellow	Seed	TVRI
BX1-166	Italia x 28/259	Yellow	Seed	TVRI
KXP-10	Royal x Amasya Siyahı	Black	Seed	TVRI

* Registration process was started for these hybrids.

Table 2. Morphologic descriptor list investigated in the study.

S/N	OIV code	IPGRI code	Vine part	Description of the character
1	001	6.1.1	Young shoot	Aperture of tip
2	003	6.1.2	Young shoot	Anthocyanin colouration on prostrate of tip
3	004	6.1.3	Young shoot	Density of prostrate hairs on tip
4	006	6.1.4	Young shoot	Density of erect hairs on tip
5	010	6.1.5	Shoot	Attitude (habit)
6	007	6.1.6	Shoot	Color of dorsal side of internodes

Table 2. Contd.

7	008	6.1.7	Shoot	Color of ventral side of internodes
8	013	6.1.8	Shoot	Color of dorsal side of nodes
9	014	6.1.9	Shoot	Color of ventral side of nodes
10	015	6.1.11	Shoot	Density of erect hairs on internodes
11	016	6.1.14	Shoot	Number of consecutive tendrils
12	017	6.1.15	Shoot	Length of tendrils
13	051	6.1.16	Young leaf	Colour of upper surface
14	053	6.1.17	Young leaf	Density of prostrate hairs between main veins
15	056	6.1.20	Young leaf	Density of erect hairs on main veins
16	065	6.1.21	Mature leaf	Size of blade
17	067	6.1.22	Mature leaf	Shape of blade
18	068	6.1.23	Mature leaf	Number of lobes
19	070	6.1.24	Mature leaf	Anthocyanin colouration of main veins
20	074	6.1.25	Mature leaf	Profile of blade in cross section
21	075	6.1.26	Mature leaf	Blistering of upper side of blade
22	076	6.1.27	Mature leaf	Shape of teeth
23	077	6.1.28	Mature leaf	Size of teeth in relation to blade size
24	078	6.1.29	Mature leaf	Ratio length/width of teeth
25	079	6.1.30	Mature leaf	Opening/overlapping of petiole sinus
26	081/2	6.1.32	Mature leaf	Tooth at petiole sinus
27	82	6.1.33	Woody shoot	Opening/ overlapping of upper lateral sinus
28	94	6.1.34	Mature leaf	Depth of upper lateral sinuses
29	301	6.1.35	Mature leaf	Density of prostrate hairs
30	087	6.1.38	Mature leaf	Density of erect hairs
31	093	6.1.40	Mature leaf	Length of petiole compared with middle vein
32	102	6.1.41	Woody shoot	Structure of surface
33	103	6.1.42	Woody shoot	Main color
34	151	6.2.1	Flower	Sexual organs
35	-	6.2.2	Berry	Size
36	204	6.2.3	Bunch	Density
37	206	6.2.4	Bunch	Length of peduncle of primary bunch
38	220	6.2.5	Berry	Length
39	223	6.2.6	Berry	Shape
40	241	6.2.7	Berry	Formation of seed
41	236	6.2.8	Berry	Color of skin
42	241	6.2.9	Berry	Intensity of the anthocyanin coloration of flesh

Table 3. DNA fragment sizes (in bp) amplified with 20 microsatellite markers in 23 hybrids and 2 varieties.

Variable	VVS1	VVS 2	VVS 3	VVS 4	VVS 5	VVS 29	VVMD 5	VVMD 6	VVMD 7	VVMD 26
7/1	170, 188	139, 151	235, 235	176, 176	117, 119	168, 168	225, 234	212, 212	254, 254	251, 253
5/2	178, 188	151, 151	235, 235	176, 176	148, 150	168, 176	225, 225	212, 212	254, 254	251, 253
70/1	178, 190	139, 153	235, 235	179, 180	117, 119	168, 176	234, 234	212, 212	254, 254	249, 253
95/3 (İsmetbey)	178, 188	139, 151	232, 232	174, 174	8, 890	168, 168	234, 236	210, 212	250, 254	249, 253
91/3 (Pembe77)	178, 182	149, 151	232, 232	176, 176	117, 119	168, 168	234, 234	210, 212	250, 258	249, 253
43/1	178, 188	151, 151	232, 232	176, 176	117, 150	168, 176	234, 236	212, 212	240, 258	253, 253
ÇH1 (Atak77)	178, 188	133, 149	230, 230	176, 186	109, 111	168, 176	234, 234	197, 212	240, 258	249, 253
130/1	178, 188	149, 149	228, 230	176, 176	113, 115	168, 168	234, 234	210, 212	240, 254	251, 253
53/1	178, 188	133, 141	232, 232	176, 176	NA	168, 176	234, 234	210, 212	250, 250	249, 253
83/1	178, 190	149, 149	228, 230	176, 176	117, 119	168, 168	234, 234	210, 212	250, 250	251, 253
85/1	178, 188	133, 133	228, 230	176, 176	148, 150	168, 168	234, 234	210, 212	250, 254	249, 253
86/1	178, 188	149, 149	230, 232	176, 176	113, 115	168, 168	234, 234	210, 212	254, 263	249, 251
7/S-176	178, 188	139, 149	232, 232	176, 176	117, 150	168, 168	234, 236	210, 212	254, 263	249, 253
26/D-3	178, 188	139, 139	232, 232	176, 176	117, 150	168, 168	225, 225	210, 212	254, 254	249, 253
16/A-101	178, 178	139, 139	230, 230	176, 176	117, 150	168, 168	236, 236	212, 212	258, 258	249, 253
15 /A- 61	178, 188	139, 153	230, 230	176, 186	148, 150	168, 168	236, 236	210, 212	258, 258	249, 253
29/C-52	178, 188	139, 139	230, 230	180, 180	117, 150	168, 168	236, 236	197, 214	260, 260	251, 253
15/B-56	178, 182	139, 139	230, 230	176, 176	117, 150	168, 168	236, 236	197, 214	258, 258	253, 253
BX2-149	182, 188	139, 139	230, 230	180, 180	148, 150	168, 176	236, 236	212, 214	254, 260	249, 253
FX1-1	178, 188	139, 151	228, 230	176, 176	117, 119	168, 168	236, 236	212, 214	258, 258	253, 253
FX1-10	178, 190	151, 151	230, 230	180, 180	117, 119	168, 176	236, 236	212, 214	240, 258	253, 253
BX1-166	178, 188	139, 153	230, 230	180, 180	148, 150	168, 176	236, 236	197, 214	254, 254	249, 253
KXP-10	178, 188	139, 139	230, 230	180, 180	113, 150	170, 176	236, 236	197, 204	258, 258	249, 253
Alfonse L.	178, 188	139, 139	230, 230	180, 180	113, 119	168, 168	236, 236	197, 214	260, 260	249, 253
Italia	178, 188	131, 141	230, 230	180, 180	NA	168, 170	236, 242	210, 214	250, 258	249, 251

	VVMD 27	VVMD 28	VVMD 31	VVMD 32	VVMD 36	VrZAG 21	VrZAG 62	VrZAG 67	VrZAG 79	VrZAG112
7/1	191, 191	267, 267	212, 212	251, 255	276, 276	202, 202	192, 195	121, 140	242, 250	232, 236
5/2	195, 195	267, 271	212, 216	255, 261, 251, 257	280, 280	202, 205	192, 195	132, 147	242, 260	232, 232
70/1	195, 195	267, 267	214, 218	251, 257	280, 280	205, 210	192, 195	125, 135	250, 260	232, 243
95/3 (İsmetbey)	185, 191	267, 267	212, 212	251, 261	276, 280	198, 202	189, 195	130, 140	250, 260	229, 238
91/3 (Pembe77)	191, 191	267, 271	212, 214	255, 261	280, 280	202, 212	195, 205	140, 140	250, 260	226, 236
43/1	191, 195	227, 247	214, 214	255, 261	NA	210, 210	195, 205	132, 132	260, 265	232, 232
ÇH1 (Atak77)	193, 193	247, 257	216, 218	255, 261	280, 280	190, 210	205, 205	132, 150	250, 260	229, 232

Table 3. Contd.

130/1	191, 193	221, 247	212, 218	251, 261	280, 280	202, 202	189, 192	130, 130	242, 250	238, 243
53/1	191, 191	235, 235	212, 212	251, 251	280, 280	190, 205	192, 205	147, 147	242, 250	226, 238
83/1	191, 191	247, 247	212, 214	251, 257	294, 294	202, 212	195, 195	132, 150	250, 260	226, 236
85/1	185, 195	247, 267	212, 218	255, 257	280, 280	205, 214	192, 205	132, 135	242, 250	226, 238
86/1	185, 185	267, 278	210, 214	257, 263	280, 280	190, 202	192, 192	132, 132	250, 255	226, 236
7/S-176	185, 185	259, 267	210, 214	257, 263	276, 276	188, 190	192, 192	132, 135	242, 260	226, 238
26/D-3	191, 191	259, 271	210, 214	255 261	NA	190, 205	192, 192	121, 140	250, 255	236, 238
16/A-101	191, 191	247, 259	214, 216	261, 271	294, 294	190, 210	192, 205	121, 132	250, 255	236, 236
15 /A- 61	185, 195	267, 267	214, 216	255, 261	NA	190, 205	205, 205	130, 132	250, 260	232, 238
29/C-52	185, 195	267, 271	212, 218	261, 271	276, 280	188, 190	192, 205	130, 132	240, 242	236, 238
15/B-56	185, 193	253, 259	216, 230	271, 271	276, 280	210, 210	192, 205	132, 152	242, 250	238, 241
BX2-149	193, 200	259, 271	212, 214	255, 261	294, 294	190, 190	192,192	130, 150	240, 250	238, 241
FX1-1	195, 200	247, 271	214, 214	257, 261	276, 276	205, 205	192, 205	132, 134	240, 250	229, 249
FX1-10	200, 200	259, 267	212, 214	257, 261	280, 280	190, 210	195, 195	132, 134	240, 250	229, 236
BX1-166	193, 193	259, 271	214, 218	257, 271	294 294	205, 214	192, 205	132, 152	242, 260	229, 243
KXP-10	191, 191	227, 271	216, 218	263, 271	294, 294	210, 210	205, 205	132, 152	245, 265	229, 249
Alfonse L.	193, 193	235, 259	212, 218	257, 263	276, 276	190, 210	189, 195	130, 150	240, 242	229, 243
Italia	191, 195	227, 259	212, 214	251, 261	276, 280	190, 205	192, 195	132, 152	250, 260	229, 243

NA: no amplification was obtained.

Hybrids and standard varieties were genotyped with 20 SSR loci including VVS1, VVS2, VVS3, VVS4, VVS5, VVS29 (Thomas and Scott, 1993), VVMD5, VVMD6, VVMD7 (Bowers et al., 1996), VVMD26, VVMD27, VVMD28, VVMD31, VVMD32, VVMD36 (Bowers et al., 1999), ssrVrZAG21, ssrVrZAG62, ssrVrZAG67, ssrVrZAG79 and ssrVrZAG112 (Sefc et al., 1999). 23 hybrids and 2 standard cultivars were analysed for the previous-mentioned SSR markers (Table 3).

PCR reactions were carried out in 25 µl of 1 x reaction buffer that contained $(NH_4)_2SO_4$, 1.5 mM of $MgCl_2$, 300 µM of each dNTP, 1 µM of primer, 1 U of Taq DNA polymerase (Fermentas) and 30 ng of genomic DNA. In the PCR reactions, predenaturation was conducted at 94°C for 3 min, and 40 cycles were applied. In each cycle, denaturation was conducted at 94°C for 30 s, annealing was conducted between 46 and 56°C for 45 s, depending on the individual primers, and extension was performed for 90 s at 72°C. The final extension stage was conducted for 7 min at 72°C. PCR-amplified DNA fragments were separated on a 3% high-resolution agarose gel with1X TBE buffer and were stained with ethidium bromide. The agarose gels were visualised on a UV transilluminator. For each variety, a score of 1 was assigned if the DNA fragment was present, and a score of 0 was applied if the DNA fragment was not observed. Based on the distance matrix constituted by NTSYSpc, a UPGMA dendrogram was constructed. The marker index for SSR markers was calculated to characterise the capacity of each primer to detect polymorphic loci among genotypes. The polymorphism information content (PIC) of the markers produced by a particular primer was calculated according to the formula: $PIC = 1-\sum pi2$, where pi is the frequency of the allele (Smith et al., 1997).

RESULTS

Ampelographic clustering

Some ampelographic observations did not show any difference for all hybrids. These are number of consecutive tendrils (OIV 016), density of erect hairs on tip (OIV 006), tooth at petiole sinus (OIV 081/2), structure of surface (OIV 102), and degree of resistance to oidium (OIV 455). The maximum difference was obtained from berry shape (OIV 223).

The UPGMA dendrogram, constructed on the basis of ampelographic scoring (0 to 9) using a distance matrix, is shown in Figure 1. Hybrids were

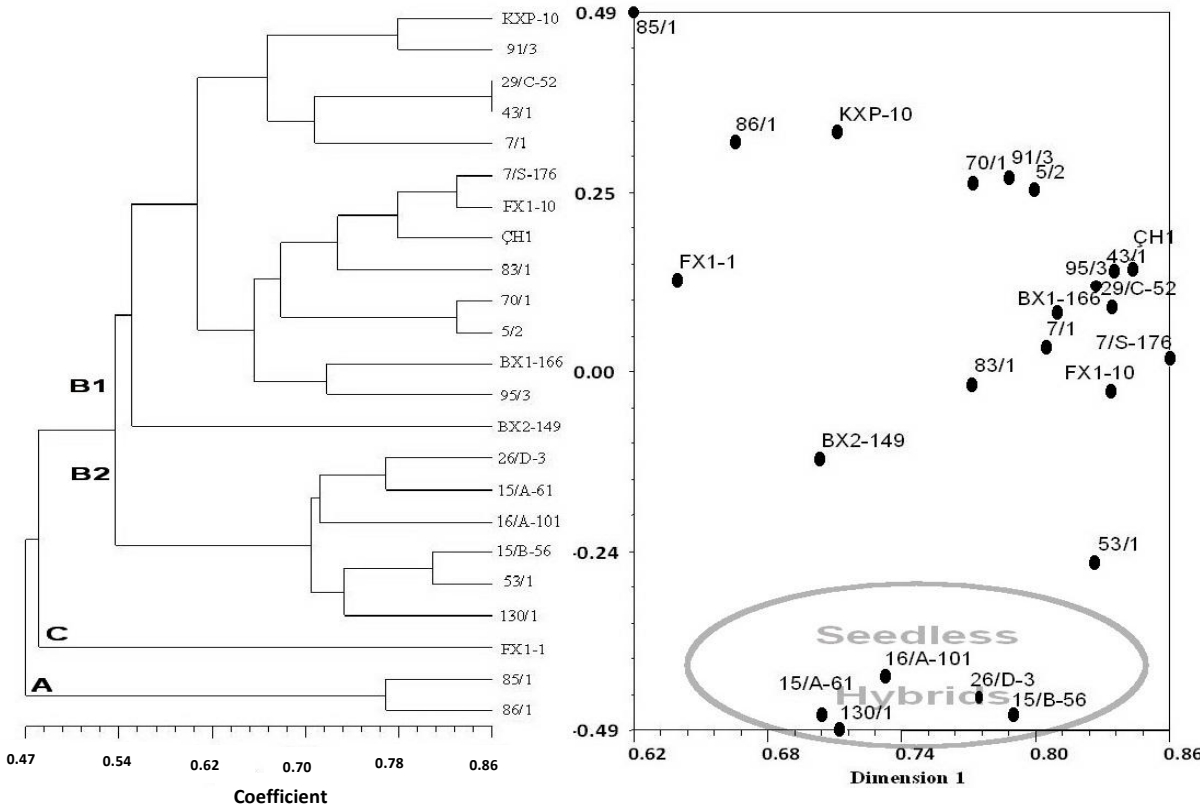

Figure 1. Genetic relationship dendrogram of hybrids (left) and principal component analyses Graph (right) based on 68 ampelographic characteristics.

were located three main groups (A, B and C) in the ampelographic dendrogram.

Also, group B separated two sub-groups (B1 and B2). Hybrids 85/1 and 86/1 (group A), which belong to AHCRI, were separated from the other hybrids (group B1, B2 and C) in the phylogenetic dendrogram. Compared to the other hybrids, hybrids 85/1 and 86/1 have very similar phenotypic characteristics. The least distance value was detected between hybrids 29/C-52 and 43/1 in group B1, even though these varieties were obtained from different institutions. While the majority of seeded hybrids was located in Group B1, all seedless hybrids was located in group B2. Also, seedless hybrids were collected together in the principal component analyses graph (Figure 1).

Only hybrid FX1-1 took part alone in group C. All hybrids have different parents except FX1-1 and FX1-10. Parents were chosen from standard table grapes by AHCRI. On the other hand, TVRI chose parents from standard table grapes and disease resistant grapes.

Molecular clustering

In the molecular analyses, twenty-three hybrids and two standard cultivars were analysed for the previous-mentioned SSR markers. DNA fragment sizes (in bp) amplified with 20 SSR markers is shown Table 3.

Depending on the hybrid and marker size of the allele ranged from 88 to 294 bp. Allele sizes are in compliance obtained in previous studies.

The UPGMA dendrogram, constructed on the basis of SSR markers scored using a distance matrix, is shown in Figure 2. Hybrids were located two main groups (A and B) in the molecular dendrogram. AHCRI hybrids and standard cultivars were located in group B except three hybrids. All TVRI hybrids were located in group A. Also AHCRI hybrids and TVRI hybrids were grouped two different areas in the principal component analyses graph (Figure 2).

Each SSR loci, primer sequence, number of total bands, average polymorphism rate (PIC) and allele size range (bp) are shown in Table 3. The closest genetic relationship was observed between 95/3 and ÇH1, which belong to AHCRI and were categorised into group B. Even if these hybrids have different ampelographic characteristics amplified gene region may have similar gene regions. In mind that we scanned the genome, a very limited area with 20 SSR markers also rate is not very high similarity between the two hybrids. Second closest genetic relationship was observed between 85/1 and 86/1, which also belong to AHRCI and were categorised into group A. These hybrids also have very similar

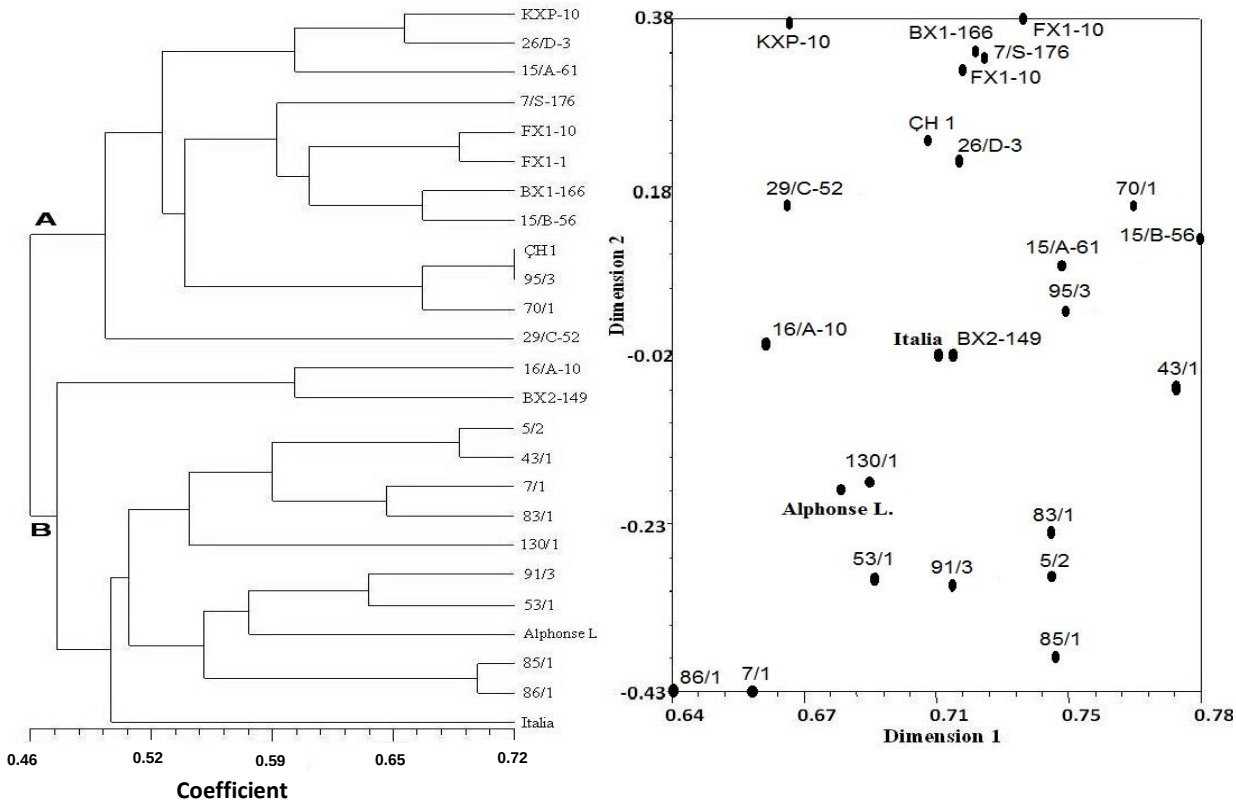

Figure 2. Genetic relationship dendrogram of hybrids (left) and principal component analyses Graph (right) based on SSR markers.

ampelographic characteristics.

Ampelographic and molecular clustering

Ampelographic and molecular characteristics were evaluated concurrently to determine interaction effects. Genetic similarity among hybrids increased when ampelographic and molecular traits were evaluated together. Similarity ratio ranged from 0.78 to 0.92.

Two different groups (A and B) were obtained, and hybrids created at different institutions were categorised into separate groups (Figure 3). Similar to the results of ampelographic characterisation, seedless varieties were clustered in group B. The closest genetic relationships were observed between hybrids 29/C-52 and 43/1, even though these varieties were obtained from different institutions. Moreover, hybrids 7/S-176 and FX1-10, which were created at TVRI, were also genetically more similar than other hybrids.

Similarly, ampelographic clustering, while all seeded hybrids was located in Group A, all seedless hybrids was located in group B except 53/1. Also, 53/1 seed structure is between stenospermocarpic seedless varieties and seeded varieties. Though this hybrid shows some berries stenospermocarpic structure, some berries shows small seed. After evaluation, it was observed that the seeded hybrids are more dominant than the seedless types. Seedless hybrids were grouped together in the principal component analyses graph (Figure 3).

DISCUSSION

Both the ampelographic and molecular characterization allowed separating hybrids. Ampelographic characters might usually be insufficient in the differentiation of closely related genotypes due to ecological factors and vine growth stages. Nevertheless, ampelographic characters are needed when describing the accessions in a gene bank to detect close agronomic mutations (Ortiz et al., 2004).

Ampelographic clustering of hybrids and cultivar candidates and the construction of the UPGMA dendrogram were based on ampelographic scoring (0 to 9). Also, these results will be used in determining the priority of registration.

Sabir et al. (2009) was obtained that similar match among seedless hybrids and hybrids with seed in UPGMA dendrogram on the basis of ampelographic data. They characterised 41 ampelographic descriptors with 44 It was also concluded that the relationship between

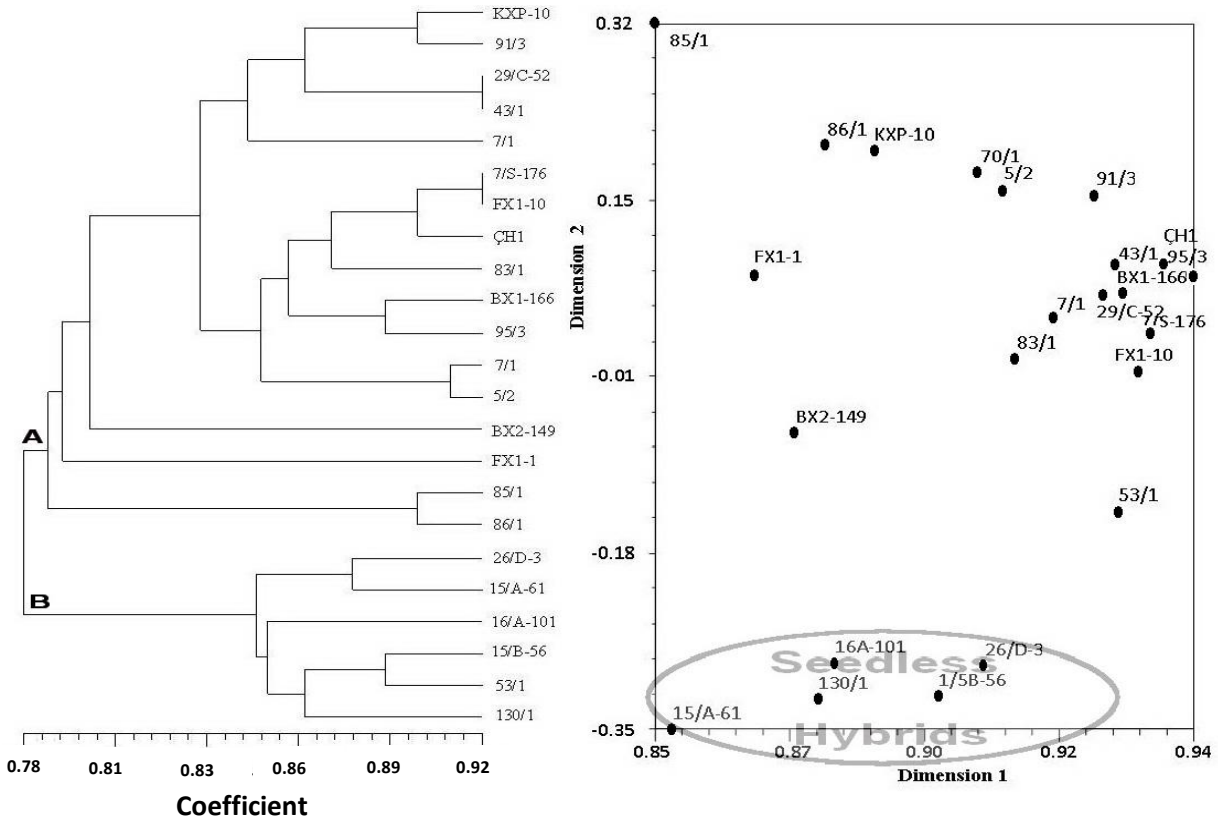

Figure 3. Genetic relationship dendrogram of hybrids (left) and principal component analyses graph (right) based on SSR markers and ampelographic characteristics.

types was highly dependent on the geographic origin of the cultivars. Twenty primers, selected on the basis of their discriminating potential, generated a total of 157 bands, of which 140 were polymorphic. The dendrogram constructed by the two approaches were largely similar in both the clustering position and divergence of varietal groups.

The size of the SSR DNA fragments produced from the hybrids was similar to those observed in previous studies. Hvarleva et al. (2004) reported similar band sizes using SSR markers in 79 Bulgarian table and wine grapes. Zulini et al. (2002) used SSR primers of VVS 2, VVMD 5, VVMD 7, VrZAG 47, VrZAG 62 and VrZAG 79 in 38 Northern Italian grapes, and reported allele sizes that were similar to those obtained in the present study (Table 3). According to the results of molecular characterisation, AHCRI and TVR hybrids were categorised primarily into two different groups (group A and B, respectively) (Figure 2).

The molecular characterisation of the hybrids revealed significant differences among hybrids with SSR markers. The closest genetic relationship was observed between 95/3 and ÇH1, which belong to AHCRI and were categorised into group B. Hybrids obtained from different institutions were well separated from each other, forming two main branches or groups (A and B) with a few exceptions. The standard varieties were categorised into group B, which included the AHCRI hybrids.

The ampelographic and phylogenetic relationship dendrogram was strongly affected by seed and seed-based characteristics. Seedless hybrids were located in the same branch (group B2) of the phylogenetic dendrogram with the exception of hybrid 53/1, which is not a completely seeded hybrid. Seedless hybrids were also clustered in the PCA graph. Similar results were reported by Sabır et al. (2009), who demonstrated that 9 out of 11 stenospermocarpic seedless varieties fell in the same cluster and the two remaining varieties were dispersed throughout the dendrogram. The shortest distance between varieties was observed between 29/C-52 and 43/1, even though they were created by different institutions.

The present study is the first comprehensive evaluation of the genetics of hybrid grapevines obtained from two different institutions. Ampelographic characterisation and the identification of SSR markers were proven to be powerful and efficient tools for the determination of cultivars and for the analysis of their genetic structure. Sixty-eight ampelographic characteristics and 20 microsatellite markers were applied, and the analysis was informative

and efficient. All of the hybrids were successfully distinguished via ampelographic and molecular characterisation; however, compared to molecular characterisation, ampelographic characterisation provided superior results because more ampelographic characteristics were determined than via molecular markers. This dominance should be taken into account when interpreting the number of characters examined.

There are differences between individuals in a very limited area in their genome. In order to find these differences, this area should be screened for a sufficient number of markers. Moreover, molecular markers only provide information on a limited region of the genome. By increasing the number of markers, the reliability of molecular characterisation can be improved and the genetic structure of the hybrids can be determined more accurately (İşçi et al., 2010; Atak and Söylemezoğlu, 2007).

Davies and Savolainen (2006) reported that commonly used biodiversity are phenotypic and genetic (genotypic) variation and the numbers of reconstructed morphological change along the branches of the phylogenetic tree (morphological branch lengths) were significantly correlated with the number of reconstructed changes in genetic characters (molecular branch lengths).

Genome mapping also needs characterization of ampelographic data and molecular data together. İşçi et al. (2010) and Gökbayrak et al. (2010) mentioned that ampelographical quantitative trait locus should be used for with molecular markers. On the other hand, for quantitative trait locus (QTL) analysis and genome we should use more ampelographic and molecular data.

Most of the hybrids have different parents though FX1-1 and FX1-10 have same parents. One of the mother or the father is common in some hybrids obtained in different Institute. Eventually, different Institute hybrids mixed in the ampelographic characteristic dendrogram. Conversely, molecular characterization showed more genetic diversity than ampelographic characterization. Also, genetic similarity among hybrids increased when ampelographic and molecular traits were evaluated together. These can be related seed-based characteristics, number of markers, different parents and ecologies.

Karataş et al. (2007) was characterized by use of six highly polymorphic microsatellite loci (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, and VrZAG79) 39 Turkish grapevine (*V. vinifera* L.). They observed similar results when analysing the dendrogram was actually the general tendency of cultivars of the same regions to group together rather than genotypes belonging to the same variety.

Ampelographic and molecular data should be evaluated together for characterization studies even if molecular characterization has more superior characteristics. Many researches were studied successfully both method for characterization of different grape cultivars (Roldán-Ruiz et al., 2001; Santiago et al., 2005; Sabır, 2009).

Studies on the genetic diversity and relatedness of varieties via molecular markers can improve the use of different genotypes in breeding programs and the design of new hybrids. In addition, the resultant DNA fingerprints can be useful in certification programs to protect new releases (Raluca et al., 2010). In this study, 23 different candidates created at two different institutions were characterised, and numerous crop characteristics were determined prior to registration. The information in the obtained database can be used for cultivar identification, parentage analysis, and legal protection.

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