Ampelographic and Molecular Diversity among Grapevine (*Vitis* spp.) Cultivars

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Abstract: This study presents the ampelographic and molecular characterization of 44 grapevine cultivars. Ampelographic data were obtained during two vegetation periods using the latest version of the descriptors. Based on the mean values transformed by the method indicated in IBPGR publications, a dendrogram was constructed. ISSR analysis was also employed to characterize the genotypes at the DNA level. Twenty primers, selected on the basis of their discriminating potential, generated a total of 157 bands, of which 140 were polymorphic. The dendrograms constructed by the two approaches were largely similar in both the clustering position and divergence of varietal groups. The least distance was observed between Yuvarlak Cekirdeksiz and Superior Seedless. The clustering position of cultivars throughout the dendrograms was basically related to the genetic distances and main uses, as well as to geographic origins.

Keywords: ampelography; genetic distance; ISSR; *Vitis* spp.

Turkey has a long history of viticulture, dating back to 3500 B.C. (Oraman & Agaoglu 1969). The country has been suggested as one of the regions where grapes were first cultivated (Winkler *et al*. 1974). Accordingly, more than one thousand grape cultivars are grown, though a few dozens of them contribute to commercial production. This wide biodiversity in grapevine germplasm provides invaluable aids to breeders. However, common vegetative propagation methods, long history of viticulture and the reliance on ampelography as a single method in taxonomic studies pose contradictions in definitions of genotypes among researchers (Thomas *et al.* 1993). Ampelography is a scientific methodology used and approved for a long time to identify grape varieties. Based on the officially defined descriptors, this method has been standardized and extended by several researchers (Galet 1985; Alleweldt & Dettweiler 1986; Santiago *et al.* 2005). Several studies in Turkey have also been conducted on the ampelographic identification of grapes using former definitions (Agaoglu *et al*. 1990; Kara 1990). To overcome the restrictions of a single methodology, further techniques that use molecular markers have been widely employed in the last decades. Recent improvements using different tools such as isoenzymes (Weeden *et al.* 1988), RFLP (Bourquin *et al*. 1993), RAPD (Ye *et al*. 1998), AFLP (Cervera *et al*. 2000; Ergul *et al*. 2006), SSR (Bowers *et al*. 1996; Sefc *et al*. 1999) have provided valuable information on biodiversity in *Vitis* material worldwide.

In Turkey, although the National Germplasm Repository Vineyard has been trying to preserve and identify the existing genotypes, this attempt might be further supported by comprehensive analysis. Also, some local cultivars such as Adana

Karasi, Adana Beyazi, Dokulgen and Tahannebi might be on the verge of disappearing, since their commercial uses are continually diminishing. Using wider groups of grape cultivars, the genetic relationship among cultivars of foreign or Turkish origin would be clarified.

The Inter Simple Sequence Repeat (ISSR) technique, composed of a microsatellite sequence between two SSR priming sites oriented on opposite DNA strands, was approved as a simple, quick and reliable tool used in various grape materials for certain purposes (Zietkiewicz *et al*. 1994; Moreno *et al*. 1998; Dhanorkar *et al*. 2005; Sabir *et al*. 2008).

This study mainly aims to (a) characterize wide groups of grapevines used for different purposes, employing ampelographic and ISSR-PCR techniques, (b) screen discriminative powers of ISSR primers across the genotypes investigated, and (c) analyze and compare the dendrograms constructed by two different approaches.

MATERIAL AND METHODS

Materials

Forty-four grapevine cultivars were analyzed to determine their ampelographic and molecular relationships (Table 1). The vines were about twenty years old and located at the Research and Implementation Area of Cukurova University (Adana), Turkey and grown under the same conditions. Cardinal, Flame Seedless, Alicante Bouschet and

Table 1. The cultivars and their basic characteristics

Cultivars	Main use	Colour	Seed	Cultivars	Main use	Colour	Seed
Adana Beyazi	T	W/G	Se	Yuvarlak Cekirdeksiz	T, R	W/G	St
Ata sarisi	T	W/G	Se	Flame Seedless	T, R	R/B	St
Beylerce	T	W/G	Se	King's Ruby	T, R	R/B	St
Dokulgen	T	W/G	Se	Pembe Cekirdeksiz	T, R	R/B	St
Muskule	T	W/G	Se	Siyah Cekirdeksiz	T, R	R/B	St
Panse Precoce	T	W/G	Se	Tekirdag Cekirdeksizi	T, R	R/B	St
Tahannebi	T	W/G	Se	Clairette	Wi	W/G	Se
Tilki Kuyrugu	T	W/G	Se	Hasandede	Wi	W/G	Se
Zevuk	T	W/G	Se	Kabarcik	Wi	W/G	Se
Razaki	T	W/G	Se	Narince	Wi	W/G	Se
Isabella	T	R/B	Se	Riesling	Wi	W/G	Se
Horoz Karasi	T	R/B	Se	Rumi	Wi	W/G	Se
Verigo	T	R/B	Se	Semillion	Wi	W/G	Se
Uslu	T	R/B	Se	Alicante Bouschet	Wi	R/B	Se
Honusu	T	R/B	Se	Bogazkere	Wi	R/B	Se
Cardinal	T	R/B	Se	Carignan	Wi	R/B	Se
Black Corinth	R	R/B	Pa	Gamay	Wi	R/B	Se
Baris	T, R	W/G	St	Kalecik Karasi	Wi	R/B	Se
Ergin Cekirdeksizi	T, R	W/G	St	Karasakiz	Wi	R/B	Se
Perlette	T, R	W/G	St	Okuzgozu	Wi	R/B	Se
Sultani Cekirdeksiz	T, R	W/G	St	Pinot Noir	Wi	R/B	Se
Superior Seedless	T, R	W/G	St	Sergi Karasi	Wi	R/B	Se

T – table, R – raisin, Wi – wine, W/G – white/green, R/B – red/black, Se – seeded, Pa – parthenocarpic, St – stenospermocarpic

Isabella (as representatives of *Vitis labrusca* L.) were chosen as common reference cultivars. The other cultivars belonged to *Vitis vinifera* L.

Ampelographic evaluation

For ampelographic characterization of cultivars, the IBPGR publication Grape Descriptors (Anonymous 1983) and the revised Descriptors for Grapevine (*Vitis* spp.) (Anonymous 1997) were followed. The latter was mostly preferred, while the former was utilized as complementary when needed. Highly discriminating characters were selected mainly according to the recommendation of the Office International de la Vigne et du Vin (OIV). Descriptors used in this study and their OIV codes are presented in Table 2.

The ampelographic observations were carried out during two consecutive vegetation periods in 2005 and 2006. The characters of represented vines were defined/measured according to OIV descriptors. The shoot tips were investigated when they were 10–30 cm long; the investigations on young leaves were recorded on the first four distal leaves; the mature leaf descriptions were made between berry set and véraison (beginning of berry maturity) on leaves above the cluster within the medium third of shoot; the clusters were measured when matured; the berry characteristics were obtained at ripening ones located in the middle of the bunches and ten average canes per variety were analyzed after the fall of leaves. Mean values of the investigations obtained during two years were transformed to numerical scales according to international descriptors. The resulting raw data were subjected to NTSYSpc 2.11V software (Rohlf 2004), using distance matrix. The clustering dendrogram was drawn with unweighted pair group of arithmetic average (UPGMA) (Rohlf 2004).

DNA extraction and ISSR analysis

For DNA isolation, young leaves were harvested from the germplasm collection. DNA was isolated according to the CTAB-based protocol (Doyle & Doyle 1990) with minor modifications (Kafkas *et al.* 2006). The amplification of ISSR loci was performed by the method developed by Zietkiewicz *et al.* (1994). Forty-four cultivars were analyzed by means of 20 ISSR polymorphic primers selected from 60 primers designed by the University of British Columbia (Vancouver, Canada). PCR reactions were carried out in 25 µl containing 10 ng genomic DNA, 75mM Tris-HCl pH 8.8, 20mM $(NH_4)_2SO_4$, 0.01% Tween 20, 2mM $MgCl_2$, 100µM each of dATP, dGTP, dCTP, dTTP, 0.2µM primer and 1 unit of Taq DNA polymerase (Fermentas). After 2 min at 94°C pre-denaturation, 40 cycles of PCR were performed (1 min at 94°C denaturation, 1 min at a primer-specific annealing temperature (between 50 and 54°C depending on the primers used), 2 min at 72°C extension) followed by 7 min 72°C final extension stage. PCR products were analyzed in 1.8% agarose gel with 1X TBE buffer. By staining with ethidium bromide, the gels were visualized on a UV transilluminator. Resulting data were scored for presence (1) or absence (0). Based on the distance matrix constituted by NTSYSpc, UPGMA dendrogram was constructed. For primers, polymorphism information content (PIC) values were calculated according to the formula: $PIC = 1 - \Sigma(P_{ij})^2$, where P_{ij} is the frequency of the i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers (Botstein *et al.* 1980). Resolving power was obtained by Prevost and Wilkinson (1999)'s equation; $RP = \Sigma Ib$, ($Ib = 1 - (2 \times |0.5 - p|)$, p represents the rate of I band in total genotypes).

RESULTS AND DISCUSSION

Ampelographic clustering of cultivars

The UPGMA dendrogram, constructed on the basis of ampelographic scoring (0–9) using a distance matrix, is shown in Figure 1. Ampelographic clustering of cultivars was predominantly in correspondence with genetic relatedness. Apart from Isabella (*V. labrusca* L.), diverged with a high distance level, *V. vinifera* L. cultivars noticeably tended to group according to the main use throughout the dendrogram. Nine stenospermocarpic seedless varieties out of 11 collected belonged to cluster (group) A, although the two remaining Baris and Perlette were dispersed. The least distance value was detected between Superior Seedless and Yuvarlak Cekirdeksiz. While the majority of table grapes inclined to settle an associate cluster (Group B), few table and wine grapes were scattered in the middle of the dendrogram. Nonetheless, no mismatch was established between cultivars of different use,

Table 2. Descriptor list investigated in the study

No.	OIV code	Vine part	Description of the character
1	OIV 001*	young shoot	form of tip
2	OIV 003*	young shoot	anthocyanin colouration of tip
3	OIV 004*	young shoot	density of prostrate hairs on tip
4	OIV 006	shoot	attitude (habit)
5	OIV 016	shoot	number of consecutive tendrils
6	OIV 051*	young leaf	colour of upper surface
7	OIV 053*	young leaf	density of prostrate hairs between veins
8	OIV 065*	mature leaf	size of blade
9	OIV 067*	mature leaf	shape of blade
10	OIV 068*	mature leaf	number of lobes
11	OIV 074	mature leaf	profile
12	OIV 076*	mature leaf	shape of teeth
13	OIV 079*	mature leaf	general shape of petiole sinus
14	OIV 081	mature leaf	tooth at petiole sinus
15	OIV 084*	mature leaf	density of prostrate hairs
16	OIV 093	mature leaf	length of petiole compared to middle vein
17	OIV 306**	mature leaf	autumn colouration
18	OIV 101**	woody shoot	section
19	OIV 102	woody shoot	surface
20	OIV 103	woody shoot	main colour
21	OIV 352	woody shoot	growth of axillary shoots
22	OIV 301*	bud	time of bud burst
23	OIV 302**	inflorescence	time of full bloom
24	OIV 151*	inflorescence	sex of flower
25	OIV 153	inflorescence	number of inflorescences per shoot
26	OIV 202	bunch	length
27	OIV 204*	bunch	density
28	OIV 502*	bunch	single bunch weight
29	OIV 303*	berry	time of véraison
30	OIV 304*	berry	time of full maturity
31	OIV 222**	berry	uniformity of size
32	OIV 223*	berry	shape
33	OIV 225*	berry	skin colour
34	OIV 231*	berry	anthocyanin colouration of flesh
35	OIV 232	berry	juiciness of flesh
36	OIV 235	berry	firmness of flesh
37	OIV 236*	berry	particular flavour
38	OIV 241	berry	presence of seeds
39	OIV 503*	berry	single berry weight
40	OIV 505	berry (must)	sugar content (%)
41	OIV 506	berry (must)	total acid content

*Recommended to use by the lists in Anonymous (1997), **chosen from the lists published in Anonymous (1983)

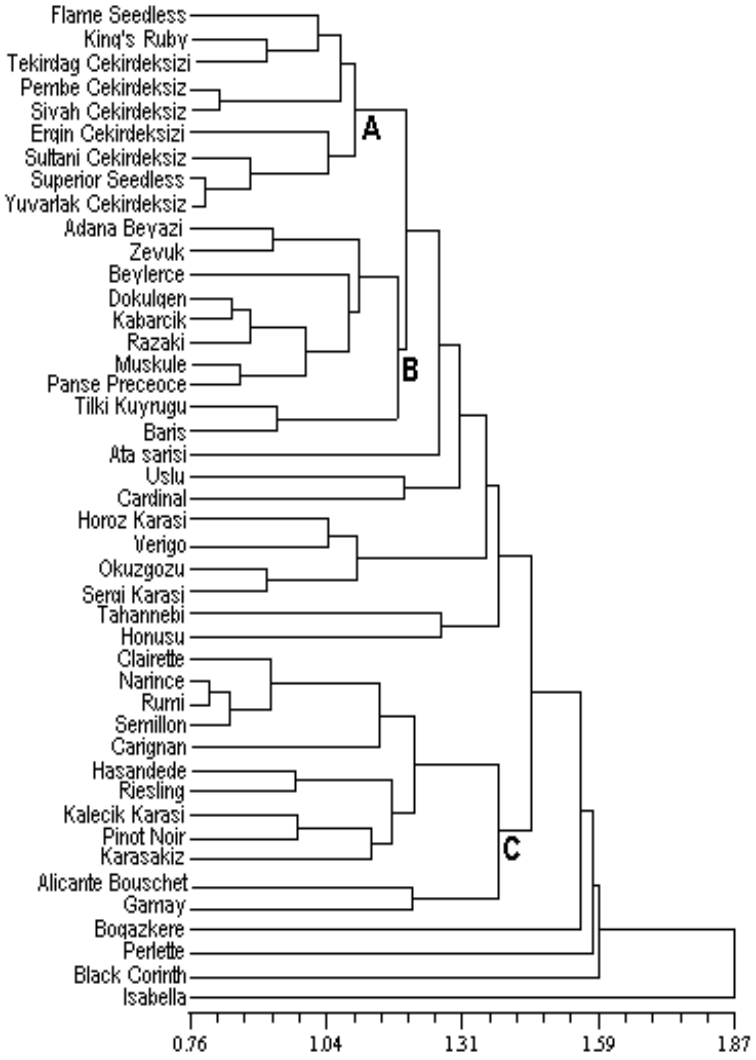

Figure 1. UPGMA dendrogram on the basis of ampelographic data

except only for Tilki Kuyrugu (table) and Baris (table/raisin). Perlette, Bogazkere and the only parthenocarpic variety Black Corinth were the most distant varieties in *V. vinifera* L. Beyond the findings mentioned above, remarkably distinguished subgroups regarding a certain cultivation area of cultivars were detected such as Adana Beyazi and Zevuk (Mediterranean), Okuzgozu and Sergi Karasi (Eastern Region). Accordingly, Tahannebi and Honusu (older cultivars in Southeast Anatolia) set up a specific pairwise location. This match was also obtained by Ergul *et al.* (2002) in a RAPD analysis of 17 indigenous cultivars. It was also concluded that the relationship between genotypes was highly dependent on the geographic origin of the cultivars. Expectedly, resulting from some of their distinguished morphological features, most wine grapes were accumulated in a subcluster (Group C). In this group, the berry colour was decisive for genotype grouping.

ISSR polymorphism

Of the 60 primers screened initially on seven cultivars, all yielded an altered interval of polymorphism from 3 to 12, with consistency to the results reported by Herrera *et al.* (2002), who detected all ISSR primers screened as polymorphic using wine grapes. Twenty primers were selected, particularly in regard to their ability to distinguish closely related cultivars. Among primers, many motifs of nucleotide repeats were realized, although the whole of four AG motifs resulted in 100% polymorphism (Table 3). Thomas *et al.* (1993) found the repeats GA and GT as the most highly represented in the *Vitis* genome, while AC repeats were more common among ISSR primers selected by using Indian grapes in a more recent study (Dhanorkar *et al.* 2005). In contrast to the studies mentioned, AT motifs in the plant kingdom have generally been approved as the most plentiful

repeat (Casasoli *et al*. 2001). Consequently, the relative abundance of nucleotide repeats in the grapevine genome indicates differences between different studies conducted on *Vitis* species.

All the primers were dinucleotide repeats, similarly to findings of Herrera *et al*. (2002), who used ISSRs in grapes. One hundred and fifty-seven bands were generated from 20 primers, out of which 140 were polymorphic with a mean percentage of 88.6%. The size of amplified fragments varied from 300 bp to 2500 bp. This interval was in agreement with the results obtained by Moreno *et al*. (1998), while it was wider than that of Dhanorkar *et al*. (2005), who reported between 300 bp and 1500 bp for different grape varieties. Total number of bands per primer ranged from 4 to 12 with an average of 7.9. Ten primers resulted in 100% polymorphism, while the lowest rate was obtained from 815 (57.1%). The RP (resolving power) value ranged from 1.272 (887) to 0.349 (858). Two primers with anchor at 5' end had the highest RPs.

ISSR clustering of cultivars

Based on a distance matrix, the dendrogram was constructed by NTSYSpc 2.11V software using UPGMA (Figure 2). Twenty primers discriminated the cultivars predominantly in accordance with their genetic relatedness and their main uses, similarly like it was in the case of ampelographic clustering. Isabella deviated with a branch at a 0.68 level. The divergence of Isabella (*V. labrusca)* might be diagnostic of a long evolutionary period in viticulture.

The clear classification of cultivars consisted mainly of three subclusters evident at an around 0.50 distance level, except five cultivars. The first class (1) comprised twelve stenospermocarpic

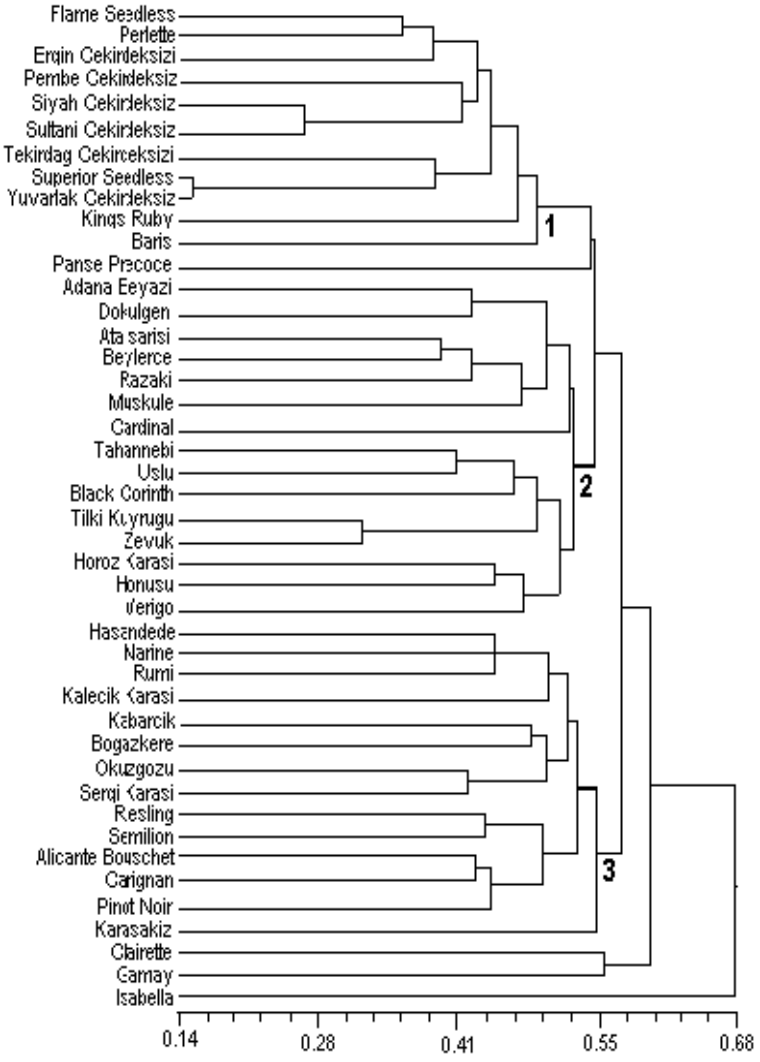

Figure 2. UPGMA dendrogram on the basis of ISSR data

Table 3. Number of total bands (NTB), number of polymorphic bands (NPB), polymorphism rate (PR), polymorphism information content (PIC) and resolving power (RP) of primers

No.	Primers	NTB	NPB	PR (%)	PIC	RP
1	UBC 808	11	11	100	0.755	0.658
2	UBC 809	8	8	100	0.844	0.661
3	UBC 810	8	7	87.5	0.648	0.987
4	UBC 814	4	3	75	0.912	0.487
5	UBC 815	7	4	57.1	0.819	0.682
6	UBC 820	6	6	100	0.895	0.691
7	UBC 823	9	8	88.9	0.475	0.906
8	UBC 824	6	6	100	0.931	0.430
9	UBC 826	7	6	85.7	0.747	0.781
10	UBC 830	4	4	100	0.762	0.715
11	UBC 834	8	8	100	0.848	0.516
12	UBC 840	8	8	100	0.821	0.602
13	UBC 846	11	10	90.9	0.795	0.694
14	UBC 848	12	12	100	0.699	0.922
15	UBC 849	6	6	100	0.833	0.616
16	UBC 857	9	9	100	0.877	0.446
17	UBC 858	7	5	71.4	0.961	0.349
18	UBC 885	9	7	77.7	0.756	0.778
19	UBC 887	10	8	80	0.518	1.272
20	UBC 888	7	4	57.1	0.507	1.184
Total		157	140	–	–	15.033
Mean		7.9	7.0	88.6	0.762	–

varieties, while the second (2) was constituted by 15 out of 17 table grapes. Wine grape varieties were gathered in the third class (3).

Among the pairs, the least distance was observed between Yuvarlak Cekirdeksiz and Superior Seedless (0.16). This relatedness was also apparent in the ampelographic analysis, despite their distinct geographic areas. Hence, this proximity could have arisen from certain associated ampelographic characters whose genetic responses were amplified by ISSRs. Most pairwise combinations were determined between 0.40 and 0.50 levels, indicating the relatively higher genetic distances of all genotypes analyzed. Based on the SSR profile of 51 Portuguese grapes, Almadanim *et al.* (2007) detected most pairs between 0.30 and 0.35 distance levels.

Table grapes largely assembled in the second class and divided further into subgroups relevant to their cultivation areas. For example, Adana Beyazi and Dokulgen, which formed a match, have been widely cultivated around the Mediterranean Region for years. Similar interpretation could be directed to the subgroup hosting Middle Anatolian varieties, namely Beylerce, Razaki and Muskule. This outcome was well suited to the findings reported by Fossati *et al.* (2001), who divided the grapes into taxonomic classes according to their geographic distribution around Italy. Kabarcik, Bogazkere, Okuzgozu and Sergi Karasi, most widely cultivated around the Eastern Region of Turkey, were hosted by an associate subgroup. Separated association drawn by external varieties was also noticeable. Another noteworthy case in

the wine grape subcluster is a tendency to group with respect to berry colour. Black varieties under the subcluster Alicante Bouschet, Carignane and Pinot Noir with pairwise Okuzgozu and Sergi Karasi were distinguished, while similar branching existed for white Hasandede, Narince and Rumi. On the other hand, Clairette and Gamay were the most distant varieties among *V. vinifera*, forming a pair.

The cultivars Cardinal, Panse Precoce, Black Corinth, Verigo and Pinot Noir, which originated outside of Turkey, showed no direct connection to any genotype, instead they were fastened to some subclusters with relatively higher distance coefficients. The most plausible interpretation upon the present case might likely be based on their external origin, with the similarity to the results obtained by Martin *et al*. (2006) on Spanish grapes.

The dendrograms constructed by two separate approaches show general similarities, especially for the grouping of some related cultivars. However, in the ampelographic dendrogram, many genotypes tended to plot outside these groups while the molecular dendrogram showed a more reasonable branching. Therefore, the results verify superiority of ISSR markers over ampelographic descriptors when the discrimination of grape cultivars is considered. Furthermore, 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).

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