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Genetic Relationship of Grape Cultivars by ISSR (Inter-Simple Sequence Repeats) Markers

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Summary

The ISSR (Inter-Simple Sequence Repeats) data were used to assess genetic relationship among 16 table grape varieties (*Vitis* ssp.) grown in Turkey. Fourteen primers selected from 50 primers generated a total of 110 clear DNA fragments in which 88 were polymorphic (80.5 %). The mean similarity index of all pairwises was 0.637, and ranged from 0.422 ('Isabella' and 'Königin der Weingärten') to 0.902 ('Alphonse Lavallée' and 'A. L. Type Royal'). Consequently, considering morphological and cultural characteristics including the high similarity value, 'Alphonse Lavallée' and 'A. L. Type Royal' could be theoretically identical. Grape cultivars analysed were divided on the clustering dendrogram (by UPGMA) according to genetic composition of related species. Our data deduced that distant genetic similarity was found between groups of different cultivars of species. 'Isabella' (*V. labrusca*) and 'Kyoho' (*V. vinifera* x *V. labrusca*, 4x) had a far similarity to *V. vinifera* grapes.

Key words. grapevine – genetic similarity – ISSR – genetic analysis

Introduction

Grapes belong to the family *Vitaceae* and the genus *Vitis* has been grouped in two sections, 11 series, and more than 60 species (Galet 1998). *Vitis vinifera* L., the most widely cultivated species of *Vitis* genus by far, is today grown throughout the temperate and tropical regions of the world for fresh fruit, raisin, juice, and wine (Winkler et al. 1974; Riaz et al. 2004). With some alterations between researchers, the accurate number of grape cultivars over the world is generally suggested around 5000 (Alleweldt 1988). In Turkey, including local cultivars nearly one thousand grapevine varieties of *Vitis vinifera* L. are grown even though nearly 10 % of them have economic importance (Agaoglu 1999). Because of its favourable climate, Turkey has had a grape industry since 3500 B.C. (Oraman and Agaoglu 1969). Thus, the country is one of the leading producers of table grape varieties. To identify the existing genetic potential, recently isozyme and DNA markers were employed by various researchers in Turkey (Agaoglu and Ergul 1999; Ergul et al. 2002; Uzun et al. 2002; Soylemezoglu et al. 2005). However none of the studies used ISSR technique for genetic analysis of *Vitis* material so far. Also, molecular marker studies based on ISSR labelling are relatively insufficient (Moreno et al. 1998; Herrera et al. 2002; Dhanorkar et al. 2005; Wu et al. 2006), while plenty of identification and diversity researches on the basis of AFLP, RAPD, RFLP and SSR (Bourquin et al. 1993; Bowers et al. 1996; Regner et al. 1996; Stavrakakis et al. 1997; This et al. 1997; Ergul et al. 2002; Fanizza et al. 2003) are available around the world.

ISSR markers, which involve PCR amplifications of DNA using a primer, composed of a microsatellite sequence anchored at 3' or 5' end by 2-4 arbitrary, could be used to assess genetic diversity (Qian et al. 2001). The technique is nearly identical to RAPD techniques except that ISSR primer sequences are designed from microsatellite regions and the annealing temperatures used are higher than those used for RAPD markers. Herrera et al. (2002) used RAPD (10 primers) and ISSR (11 primers) techniques to analyse genetic variation between four most widely planted wine grape cultivars in Chile. Researchers found two techniques able to distinguish differences in cultivars while the resolving power of ISSR profiles was higher than that of RAPDs and they recommended the latter for use a wider range of cultivars.

The objectives of the present study were to (1) reveal the ISSR-based genetic diversity among different type flavour grapes (2) analyse polymorphism level of ISSR primers used, and (3) assess the genetic closeness within some cultivars of international and Turkish interest.

Material and Methods

The young leaves of sample materials were gathered from the germplasm collection of Research and Application Vineyards of Cukurova University. The commercial varieties and their main properties used were presented in Table 1.

Grapevine genomic DNA was purified according to the CTAB-based protocol of Doyle and Doyle (1990) with minor modifications improved by Kafkas et al. (2006). The amplification of ISSR loci was performed by the method Zietkiewicz et al. (1994) with minor modifications carried out by Kafkas et al. (2006). Sixteen cultivars were analyzed via 14 polymorphic primers selected from 50 ISSR

Table 1. List of the grape cultivars studied and their main properties.

No	Cultivar	Origin	Genetic composition	Colour	Flavour
1	'Tarsus Beyazi'	(a local cultivar)	*V. vinifera*	white	neutral
2	'Italia'	'Bicane' x 'Muscat Hamburg'	*V. vinifera*	white	muscat
3	'Königin der Weingärten'	'R. Elisabet' x 'Perle von Csaba'	*V. vinifera*	white	muscat
4	'Muscat of Alexandria'		*V. vinifera*	white	muscat
5	'Perle von Csaba'		*V. vinifera*	white	muscat
6	'Razaki'		*V. vinifera*	white	neutral
7	'Yalova Incisi'	'Hoenuesue' x 'S.Gemre'	*V. vinifera*	white	neutral
8	'Muscat Hamburg'		*V. vinifera*	black	muscat
9	'Kyoho' (tetraploid)	'I. Wase' x 'Centennial'	*V. vinifera x V. labrusca*	black	foxy
10	'Red Globe'	'Hoenuesue' x 'Emperor'	*V. vinifera*	black	neutral
11	'Adana Karasi'	(a local cultivar)	*V. vinifera*	black	neutral
12	'Alphonse Lavallée'		*V. vinifera*	black	neutral
13	'Early Cardinal'	'F. Tokay' x 'A. Lavallée'	*V. vinifera*	black	neutral
14	'Isabella'		*V. labrusca*	black	foxy
15	'A. Lavallée Type Royal'		*V. vinifera*	black	neutral
16	'Trakya Ilkeren'	'A. Lavallée' x 'Perlette'	*V. vinifera*	black	neutral

primers designed by University of British Columbia. Amplification reaction was carried out in 25 µl containing 10 ng genomic DNA, 75 mM Tris-HCl pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.1 % Tween 20, 2 mM $MgCl_2$, 100 µM each of dATP, dGTP, dCTP, dTTP, 0.2 µM primer and 1 unit of DNA Taq 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 50–54 °C depending on the primers used), 2 min at 72 °C extension) followed by 7 dk 72 °C final extension stage.

Aliquots of ISSR products were analysed in 1.8 % agarose gel with 0.5X TBE buffer (40 mM Tris-acetate and 1 mM EDTA, pH 8). After staining with ethidium bromide the gels were photographed on a UV transilluminator.

ISSR data were scored for presence (1), absence (0) and each band was regarded as a locus. Jaccard similarity matrix was constructed by NTSYSpc 2.11e software. Based on the similarity matrix, a dendrogram showing the genetic relationships between genotypes was constructed using the Unweighted Pair Group Method with Arithmetic average (UPGMA). Polymorphic Information Content (PIC) values were calculated for each ISSR primer 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;

$RP = \Sigma\ Ib$ [$Ib = 1-(2\ X\ |0.5-p|)$, p represents the rate of I band in total genotypes].

Results and Discussion

Polymorphism

Data obtained from the analysis of 16 grape cultivars (*V. vinifera, V. labrusca* or interspecific hybrids) of international and Turkish interest were fingerprinted by 14 ISSR primers in order to reveal and verify genetic relationship among some aromatic and neutral grapes. A total of 110 strong and well resolved bands were generated by 14 ISSR primers (Table 2). 88 bands were polymorphic and the polymorphism rate was 80.5 %. The number of total bands for each primer ranged from 4 to 11 with the mean value of 7.86. In the previous studies usually 2 to 13 bands per ISSR primer were reported (Moreno et al. 1998; Herrera et al. 2002; Wu et al. 2006). All primers

Table 2. Number of Total Bands (NTB), Number of Polymorphic Bands (NPB), Polymorphism Rate (PR), Polymorphism Information Content (PIC) and Resolving Power (RP) of ISSR Primers used.

No	Primer	NTB	NPB	PR	PIC	RP
1	UBC 808	11	9	81.8	0.675	0.861
2	UBC 809	8	7	87.5	0.686	0.979
3	UBC 810	9	8	88.9	0.549	1.172
4	UBC 815	6	3	50.0	0.837	0.792
5	UBC 823	8	7	87.5	0.688	0.946
6	UBC 824	5	5	100.0	0.723	0.925
7	UBC 826	6	5	83.3	0.709	1.063
8	UBC 830	4	3	75.0	0.664	1.083
9	UBC 840	8	8	100.0	0.680	0.968
10	UBC 846	10	7	70.0	0.596	1.143
11	UBC 848	11	9	81.8	0.635	1.125
12	UBC 857	7	7	100.0	0.749	0.833
13	UBC 887	11	6	54.5	0.536	1.125
14	UBC 888	6	4	66.7	0.486	1.208
Total		110	88	–	–	14.223
Mean		7.86	6.29	80.5	0.658	–

Table 3. Jaccard genetic similarity index between grape cultivars.

Cvs*	TB	IT	KW	MA	PC	RA	YI	MH	KY	RG	AK	AL	EC	IS	RO	TI
TB	1.000	0.674	0.750	0.711	0.611	0.713	0.674	0.615	0.542	0.688	0.681	0.659	0.702	0.510	0.688	0.722
IT		1.000	0.710	0.663	0.598	0.693	0.625	0.688	0.524	0.613	0.621	0.654	0.650	0.435	0.696	0.587
KW			1.000	0.726	0.690	0.810	0.683	0.583	0.582	0.703	0.647	0.632	0.745	0.422	0.673	0.729
MA				1.000	0.625	0.763	0.705	0.632	0.531	0.708	0.633	0.650	0.698	0.461	0.694	0.717
PC					1.000	0.624	0.604	0.550	0.500	0.640	0.569	0.587	0.680	0.495	0.611	0.663
RA						1.000	0.735	0.647	0.631	0.755	0.670	0.696	0.763	0.462	0.722	0.729
YI							1.000	0.643	0.561	0.667	0.627	0.660	0.724	0.462	0.670	0.708
MH								1.000	0.553	0.598	0.622	0.708	0.653	0.531	0.719	0.602
KY									1.000	0.575	0.505	0.529	0.556	0.522	0.524	0.520
RG										1.000	0.663	0.663	0.710	0.449	0.689	0.694
AK											1.000	0.673	0.653	0.505	0.700	0.636
AL												1.000	0.755	0.458	0.902	0.704
EC													1.000	0.495	0.747	0.755
IS														1.000	0.472	0.461
RO															1.000	0.768
TI																1.000

***TB**: 'Tarsus Beyazi', **IT**: 'Italia', **KW**: 'Königin der Weingärten', **MA**: 'Muscat of Alexandria', **PC**: 'Perle von Csaba', **RA**: 'Razaki', **YI**: 'Yalova Incisi', **MH**: 'Muscat Hamburg', **KY**: 'Kyoho', **RG**: 'Red Globe', **AK**: 'Adana Karasi', **AL**: 'Alphonse Lavallée', **EC**: 'Early Cardinal', **IS**: 'Isabella', **RO**: 'Alphonse Lavallée Type Royal', **TI**: 'Trakya Ilkeren'

used in this study generated polymorphic bands between grape cultivars. The highest polymorphism rates were obtained from UBC 824, UBC 840 and UBC 857 with 100 %. Polymorphism percentages varied from 50.0 to 100 % and 3 to 9 polymorphic bands were detected for each primer. The average PIC was 0.658, while the lowest and highest PIC values were 0.486 (UBC 888) and 0.837 (UBC 815), respectively. In a similar study, Wu et al. (2006) generated a total of 105 bands with 91 % polymorphism rate, analysing 15 cultivars belonging to *V. amurensis, V. vinifera* and hybrids by fifteen ISSR primers. The researchers also reported a polymorphism range between 60 and 100%. In the same manner, Dhanorkar et al. (2005) were obtained a high polymorphism rate (96 %) in a genetic assessment study which included *V. vinifera, V. labrusca* and *V. rotundifolia* cultivars with 13 selected ISSR primers among 43 cultivars. These results suggest that ISSR primers are powerful and reliable tool for differentiating grape cultivars.

Genetic Similarity

ISSR genetic similarity values ranged from 0.422 for the most distant related cultivars to 0.902 for the closest ones while the average value was 0.637 (Table 3). The highest genetic similarity was found between 'Alphonse Lavallée' and 'A. L. Type Royal' (0.902), while the lowest genetic similarity was observed between 'Isabella' (*V. labrusca*) and 'Königin der Weingärten' (*V. vinifera*) with a relatively low numerical value of 0.422. The similarity value between two Muscat grapes 'Königin der Weingärten' and 'Muscat of Alexandria' was 0.726. The most distant muscat grape pairs were 'Italia' and 'Perle von Csaba' (0.598). *V. labrusca* grape 'Isabella' and the hybrid cultivar 'Kyoho' (*V. Vinifera* x *V. labrusca*) had lower similarity indexes to *V. vinifera* grapes.

Cluster Analysis

The clustering dendrogram constructed by UPGMA method computed via NTSYSpc 2.11e software was depicted in Fig. 1. Sixteen grape cultivars on the basis of ISSR fingerprinting were clearly separated from each other with exception of 'Alphonse Lavallée' and 'A. L. Type Royal' match (Fig. 2). From the ampelographic point of view, 'Alphonse Lavallée' shows the strongest similarity to 'A. L. Type Royal' outside the cultivars analysed. Furthermore, the latter is known as bud sport of 'Alphonse Lavallée'. Therefore, these two genotypes could be considered genetically identical.

'Isabella', belonging to species *V. labrusca,* appeared to be the most diverged cultivar, separated from the rest of the tree by a branch. Actually, two varieties 'Isabella' and 'Kyoho' were completely outbranched. Varieties belonging to *V. vinifera* appeared to be more distinct and separated further into several subgroups. Two muscat flavoured cultivars 'Muscat Hamburg' and 'Italia' ('Bicane'x 'Muscat Hamburg') made a pairwise combination, separated from the rest. This relation could be relevant to parental statute of these genotypes. The present case is similar to findings obtained by Sefc et al. (1997) who interpreted the SSR-based close connection between 'Cabernet Sauvignon' and 'Cabernet Franc' as linked with morphological characteristics. However might it be very surprising, Herrera et al. (2002) repudiated those assertion by RAPD fingerprinting in which different patterns were indicated between 'C. Sauvignon' and 'C. Franc'. Possible explanation upon these contradictions lie in differences for primer amplification regions throughout the *Vitis* genome. On the other hand, 'Razaki' displayed a close relation to 'Königin der Weingärten' unexpectedly, with high similarity degree (0.810). This fact might be arisen from basic of ISSR primer which recognise the short repeats

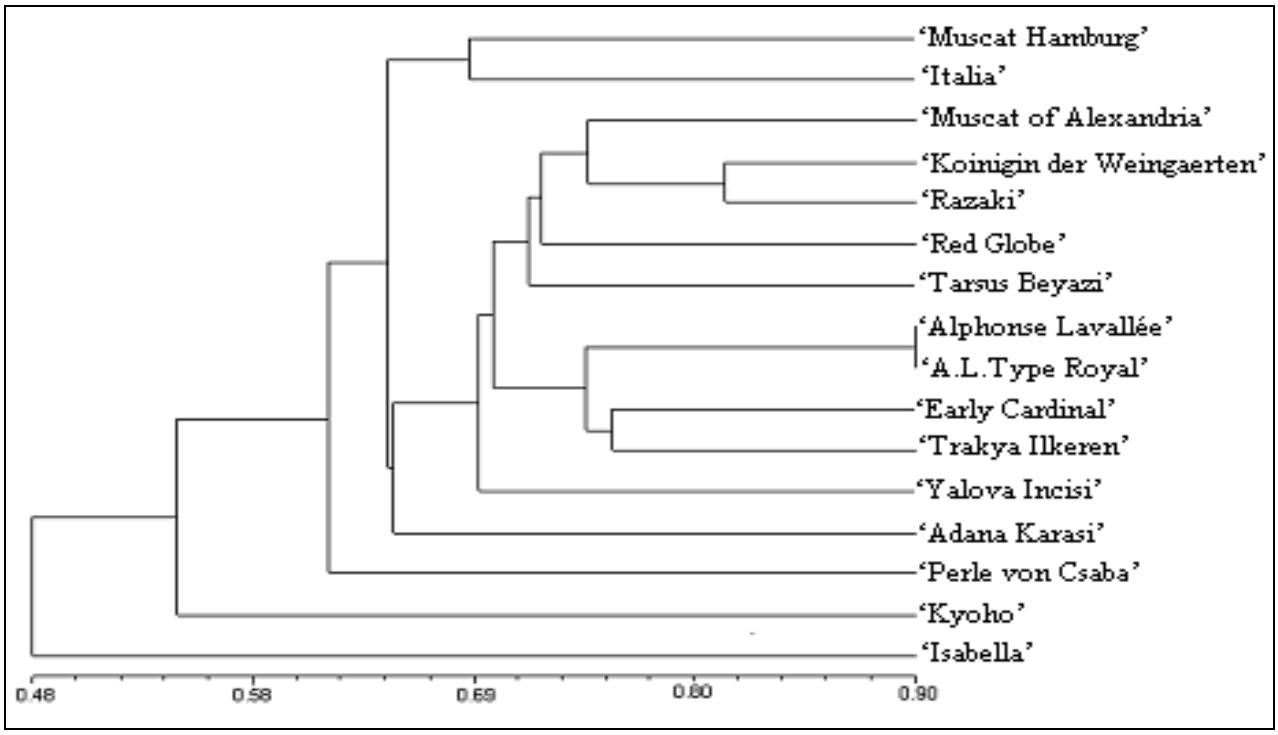


Fig. 1. UPGMA dendrogram of 16 *Vitis* varieties on the basis of ISSR data.

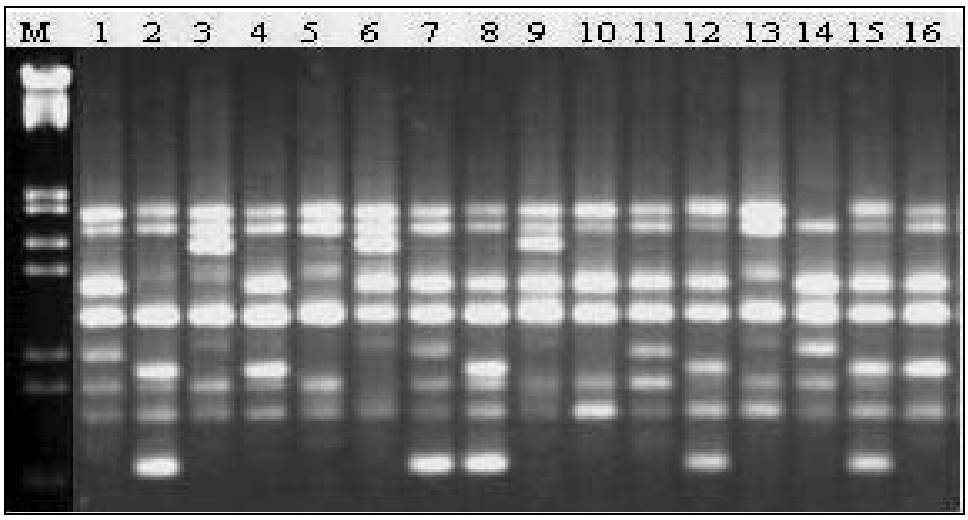


Fig. 2. DNA fingerprinting of sixteen grape cultivars using UBC 808. Numbers represent the cultivar code listed in Table 1 and M indicates DNA ladder.

throughout the genome. The last pairwise formed between 'Early Cardinal' and 'Trakya Ilkeren' indicating a close similarity (0.755). 'Perle von Csaba', the other cultivar analysed, exhibited distant similarity with a separate branch encircling the other *V. vinifera* cultivars.

Conclusion

It can be concluded that distant genetic similarity was found mainly between different cultivars of the species. 'Isabella' and 'Kyoho' had a far similarity to *V. vinifera* grapes. This outcome could be arisen from distinction in species. Although dendrogram reflects certain slight closeness according to aromatic compound, this case could not be interpreted in definite as evidence about genetic relationship related to aromatic components of genotypes. But the absolute knowledge in genetic connection upon aromatic scope of genotypes would be clarified by further studies consisting of analysis of different chemicals responsible for the taste and flavour of grapes. By using wider groups of grape cultivars with higher number of primers, genetic relationship among cultivars of foreign or Turkish interest would be further clarified. To generalize, our findings approved the ISSR technique as a powerful tool in the analysis of grape germplasm and taxonomic investigation of genetic relationship in *Vitis* material as many researchers stated before.

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