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# Genetic relationships among grapevine cultivars native to Croatia, Greece and Turkey

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## Summary

**Three sets of grapevine cultivars were analysed: (1) 6 Croatian cultivars from the island of Hvar, (2) 5 Greek cultivars from the island of Paros and (3) 9 Turkish cultivars from the region of Anatolia. These cultivars were assayed by molecular markers (RAPDs with 8 primers and SSRs on 8 loci) and analyzed in terms of genetic similarity. Cluster analysis based on Dice genetic similarity indices resulted in dendrograms using two types of data. The cultivar DNA profiles showed that there were no synonyms among the groups of cultivars tested. Cluster analysis did not point to any particular relationship among cultivars from different regions, although AMOVA analysis showed greater genetic similarity between Greek and Croatian cultivars in contrast to Turkish cultivars.**

K e y w o r d s : SSR, RAPD, genetic relatedness, cultivar introduction.

## Introduction

Most contemporary grapevine (*Vitis vinifera* L.) cultivars are fairly old and of unknown genetic background. Grapevines have been vegetatively propagated in Croatia for centuries and the introduction and spread of cultivars in Croatia is thought to have occurred in the past. This hypothesis raises the chances of Croatia's present cultivars being genetically similar to the cultivars from the countries that have in the past settled or conquered the today's Croatian area.

According to various theories ancient Greeks from the island of Paros are said to have established one of their colonies at the site of today's town of Stari Grad on the island of Hvar 2400 years ago and named it Faros after their home island (Suić 2003). Despite the lack of firm evidence about their precise origin, there are many archeological traces of the ancient Greeks' presence on the island of Hvar. The genotypes of a vegetatively propagated species, such as grapevine, could corroborate that.

The influence of Turkey (the Ottoman empire) on the entire Balkan peninsula was intense and long-lasting in the nearer past. Archeological and historical research in Anatolia in Turkey proves that this region was very important for the history of viticulture (Gorny 1996).

In this study a random sample of 9 cultivars from Turkey (the region of Anatolia), 5 most widely spread samples from the island of Paros in Greece and 6 samples of native cultivars known to be grown only on the island of Hvar in Croatia were analyzed using Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR) markers in order to determine the potential synonyms and genetic relatedness among them. The analysis of the microsatellite (SSR) profiles facilitates determining the relatedness between the cultivars and their origin (Sefc *et al*. 2000; Piljac *et al.* 2002; Maletić *et al.* 2004). This is due to the fact that the codominant markers SSRs have the potential of determining the "parent-offspring" relationships while RAPD markers can detect variation on clone level.

## Material and Methods

Young leaves were taken from cultivars listed in Tab. 1. All samples were taken from standard vineyards, not the official collections, by ampelographers or by viticulturists. The samples from Paros (Greece) were collected at the beginning of the vegetation season (early June) in local vineyards and the affiliation of samples to the cultivar name was based upon the vineyard data and the features of young leaves. Cultivar samples from Croatia and Turkey were collected from vines that were positively identified in previous years.

Young leaves picked in the spring of 2003 were lyophilized and stored at -80 °C. The DNA extraction method (Doyle and Doyle 1990) was conducted using 2 % CTAB. The method was slightly modified by adding 6 % PVP to the extraction buffer. The DNA concentration was checked by band confrontation with λDNA on 0.8 % agarose gels.

PCR amplification for RAPD analysis was carried out in 25 µl of the reaction mix containing 10 ng of template DNA, PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.8), 0.2 µM of primer, 0.1 mM of each dNTP, 1.5 mM $MgCl_2$, 1U of thermostable Taq polymerase (Sigma) and stabilized with 20 µg BSA. RAPD primers were identical with those used previously by Vokurka *et al.* (2003), the DNA was amplified in PTC-100 thermal cycler (MJ Research) with one step of 92 °C for 60 s, followed by 40 cycles of 60 s at 92 °C for denaturation, 60 s at 36 °C for annealing and 120 s at 72 °C for extension. The amplification products were separated in 1.2 % agarose gels at 120 V for 2.5 h.

Eight microsatellite loci were analysed: VVS2 (Thomas and Scott 1993), VVMD5 (Bowers *et al.* 1996), VVMD25,

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Table 1

Basic descriptive characteristics for 9 Turkish, 6 Croatian and 5 Greek grapevine cultivars

| Cultivar | Sampling location | Country | Utility | Color of skin | Berry shape | Bunch density | Total acids | Sugar content |
|---|---|---|---|---|---|---|---|---|
| Kişmiş | Olur | TR | table grape | Green-yellow | reverse oval | dense | low | high |
| Hatun parmağı | Olur | TR | table grape | Green-yellow | cylyndrical | medium dense | low | high |
| Kabarcik | Olur | TR | table grape | Green-yellow | roundish | medium | low | high |
| Al üzüm | Erzincan | TR | table grape | Red | roundish | medium dense | low | high |
| Karaerik | Olur | TR | table grape | Dark red-violet | roundish | very dense | high | medium |
| Pirtik | Olur | TR | table grape | Green-yellow | wide oval | medium | low | high |
| At memesi | Olur | TR | table grape | Dark red-violet | oval | dense | high | medium |
| Beyaz üzüm | Olur | TR | table grape | Green-yellow | roundish | very dense | low | high |
| Kara | Olur | TR | table grape | Dark red | roundish | medium dense | low | medium |
| Drnekuša type I | Hvar | HR | wine grape | Dark red | roundish | medium | low | medium |
| Drnekuša type II | Hvar | HR | wine grape | Dark red light | roundish | medium | low | medium |
| Bogd. type I | Hvar | HR | wine grape | Green yellow | roundish | medium | low | medium |
| Bogd. type II | Hvar | HR | wine grape | Green yellow | roundish | medium | low | medium |
| Prč | Hvar | HR | wine grape | Green yellow | roundish | medium | low | high |
| Kuč | Hvar | HR | wine grape | Green yellow | roundish | medium | low | low |
| Aidani | Paros | GR | wine grape | Red | cylyndrical | - | - | - |
| Mantilaria | Paros | GR | wine grape | Dark red | roundish | - | - | - |
| Monemvasia | Paros | GR | wine grape | Green yellow | oval | - | - | - |
| Aetonychi | Paros | GR | wine grape | Red | roundish | - | - | - |
| Vaftra | Paros | GR | wine grape | Red | roundish | - | - | - |

- data not available

VVMD27, VVMD28, VVMD32 (BOWERS *et al.* 1999), ssrVrZAG62 and ssrVrZAG83 (SEFC *et al.* 1999). The DNA was amplified in volumes of 25 µl containing 10 ng of template DNA, PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.8), 0.2 mM of each, forward and reverse primer, 0.2 mM of each dNTP, 2.0 mM $MgCl_2$, 1U of termostable Taq polymerase (Sigma) and stabilized with 20 µg BSA.

Electrophoresis was done on EL-800 Precast SpreadexTM gels in 30mM TAE buffer using SEA 2000 chambers (Elchrom Scientific) at 55 °C and 92 V ranging in running time from 1.5 h for smaller fragments (150 bp) up to 3 h for longer fragments (260 bp). Gels were stained by SYBR® Gold (Molecular Probes) and photographed using Polaroid® film type 667.

Genetic distance calculations based on separate RAPD and SSR data were run as described by PEJIĆ *et al.* (1998) and computed using NTSYS-pc software (ROHLF 1990). AMOVA analysis (EXCOFFIER *et al.* 1992) was based on the procedure used in the similar study (BELAJ *et al.* 2002) and was done using the Arlequin software (SCHNEIDER *et al.* 2000).

## Results and Discussion

The basic descriptive characteristics for all studied cultivars are given in the Tab. 1. Eight RAPD primers generated informative profiles with an average of 5 polymorphic bands per primer. Only well-defined bands were taken into account. Based on a total of 42 RAPD polymorphic fragments each cultivar showed a unique RAPD profile. Eight SSR primers generated codominant profiles shown in Tab. 2. The dice genetic distances among all possible pairs of cultivars were calculated from both the RAPD and the SSR data using the UPGMA algorithm and NTSYS-pc software clustered dendograms as depicted in Figs. 1 and 2.

Hierarchical analysis of molecular phenotypic diversity based on combination of the RAPD and the SSR data using AMOVA was performed to analyze the partitioning of molecular variation in the grapevine cultivars among and within countries (Croatia, Greece and Turkey) (Tab. 3). Most of the genetic diversity was attributable to the differences among the cultivars within countries (82.07 %), significant inter-population distance average (Φ-value) among zones ($\Phi_{st}$=0.179; $p < 0.001$) suggested the existence of phenotypic differentiation. $\Phi_{st}$ values between each pair of countries were mostly significant in all cases except for Greece and Croatia (Tab. 4), suggesting that the cultivars from these two countries are more closely related to each other. The dendrograms based on the Dice genetic similarities computed from both the RAPD and the SSR data also support the conclusion that the genetic differences of the Turkish cultivars are very high when compared to those from Greece and Croatia. The majority of Turkish cultivars forms specific clusters which are separate from the Croatian and the Greek cultivars, which did not form separate clusters (Figs. 1 and 2).

To test this result the SSR data from this research were joined with the SSR data from 87 additional Croatian cultivars (data not shown) and a larger dendrogram was constructed (data not shown). The Greek cultivars from this study were equally scattered in the dendrogram, while the Turkish sam-

T a b l e 2

Genotypes at 8 SSR loci for 9 Turkish, 6 Croatian and 5 Greek grapevine cultivars

| Cultivar | VVS2 | | VVMD5 | | VVMD25 | | VVMD27 | | VVMD28 | | VVMD32 | | VrZAG62 | | VrZAG83 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 Kişmiş | 135 | 141 | 240 | 246 | 243 | 243 | 185 | 194 | 251 | 271 | 251 | 257 | 189 | 203 | 193 | 197 |
| 2 Hatun parmağı | 135 | 141 | 240 | 246 | 243 | 243 | 185 | 194 | 251 | 271 | 251 | 257 | 189 | 203 | 193 | 197 |
| 3 Kabarcik | 135 | 141 | 240 | 240 | 243 | 243 | 185 | 194 | 251 | 271 | 251 | 257 | 189 | 203 | 193 | 197 |
| 4 Al üzüm | 133 | 141 | 236 | 246 | 243 | 243 | 185 | 194 | 261 | 271 | 273 | 273 | 201 | 203 | 191 | 193 |
| 5 Kara erik | 141 | 151 | 234 | 234 | 259 | 259 | 185 | 194 | 247 | 247 | 257 | 257 | 205 | 205 | 197 | 197 |
| 6 Pirtik | 143 | 143 | 236 | 246 | 243 | 245 | 185 | 194 | 271 | 271 | 273 | 273 | 195 | x | 191 | x |
| 7 At memesi | 141 | 145 | 236 | 236 | 243 | 243 | 185 | 194 | 247 | 271 | 257 | 273 | 201 | 205 | 197 | 197 |
| 8 Beyaz üzüm | 135 | 135 | 236 | 236 | 243 | 253 | 183 | 185 | 239 | 251 | 273 | 273 | 189 | 189 | 191 | 197 |
| 9 Kara üzüm | - | - | - | - | 243 | 253 | 185 | 185 | 239 | 239 | 273 | 273 | 189 | 189 | 191 | 197 |
| 10 Drnekuša type I | 133 | 145 | 228 | 240 | 245 | 259 | 179 | 194 | 251 | 261 | 257 | 273 | 189 | 191 | 193 | 197 |
| 11 Drnekuša type II | 143 | 145 | 226 | 228 | 243 | 245 | 179 | 179 | 251 | 261 | 257 | 257 | 191 | 205 | 197 | 197 |
| 12 Bogdanuša type I | 143 | 151 | 222 | 228 | 245 | 267 | 183 | 194 | 251 | 261 | 251 | 257 | 189 | 191 | 193 | 197 |
| 13 Bogdanuša type II | 143 | 151 | 222 | 228 | 245 | 267 | 183 | 194 | 251 | 261 | 251 | 257 | 189 | 191 | 193 | 197 |
| 14 Prč | 133 | 151 | 226 | 228 | 243 | 253 | 183 | 194 | 261 | 275 | 273 | 273 | 195 | 205 | 191 | 197 |
| 15 Kuč | 133 | 145 | - | - | 245 | 245 | 189 | 189 | 261 | 261 | 273 | 273 | 189 | 197 | 197 | 197 |
| 16 Aidani | 133 | 135 | 240 | 240 | 245 | 247 | 179 | 194 | 139 | 247 | 257 | 257 | 189 | 205 | 193 | 197 |
| 17 Mantilaria | 145 | 145 | - | - | 247 | 259 | 179 | 179 | 257 | 261 | 265 | 265 | 197 | 203 | 191 | 203 |
| 18 Monemvasia | 133 | 141 | 226 | 234 | 243 | 253 | 175 | 179 | 247 | 261 | 241 | 251 | 189 | 197 | 191 | 197 |
| 19 Aetonychi | 133 | 145 | 234 | x | 243 | 249 | 179 | 185 | 251 | 251 | 273 | 273 | 189 | 189 | 193 | 197 |
| 20 Vaftra | - | - | 226 | 226 | 249 | 259 | 179 | 183 | 257 | 257 | 265 | 265 | 197 | 205 | 191 | 197 |

x = very faint bands (scored as missing data)
- = missing data.

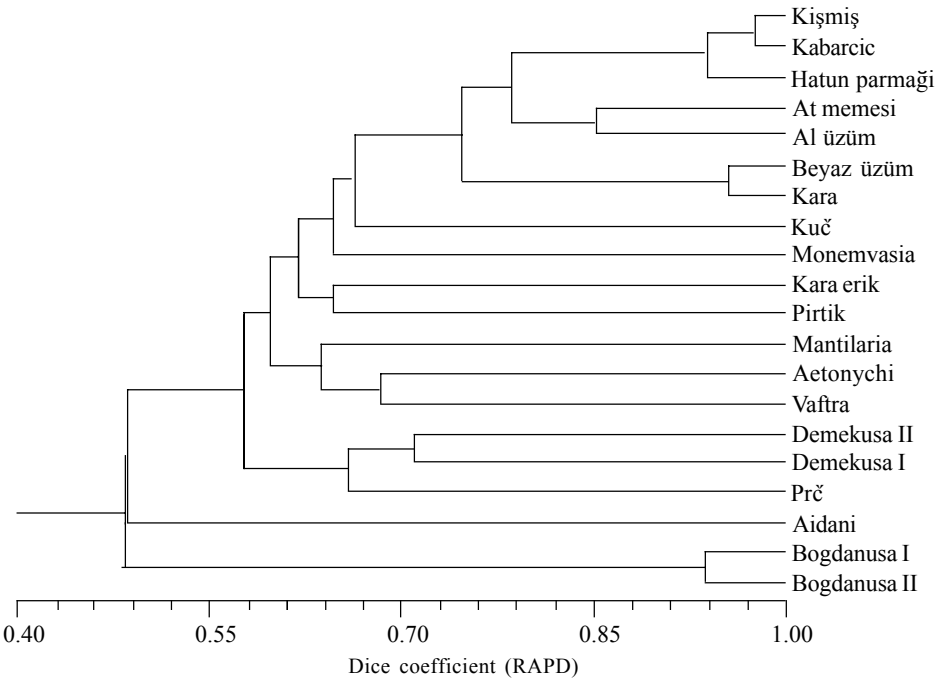


Fig. 1: Dendogram based on Dice genetic similarities among 6 Croatian (HR), 5 Greek (GR) and 9 Turkish (TR) grapevine cultivars, computed from RAPD data generated by 8 primers.

ples were clearly separated in one individual cluster, supporting the results of the AMOVA analysis and the dendrograms shown in this study (Figs. 1 and 2).

The native vegetatively propagated varieties have the potential to be very old genotypes and, considering the isolated position and the viticultural tradition of the Mediterranean islands, they might represent the genotypes that may be several centuries, even millenniums, old. The idea of this study was to provide the evidence of introducing wine grapes that might support the assumption that the island of

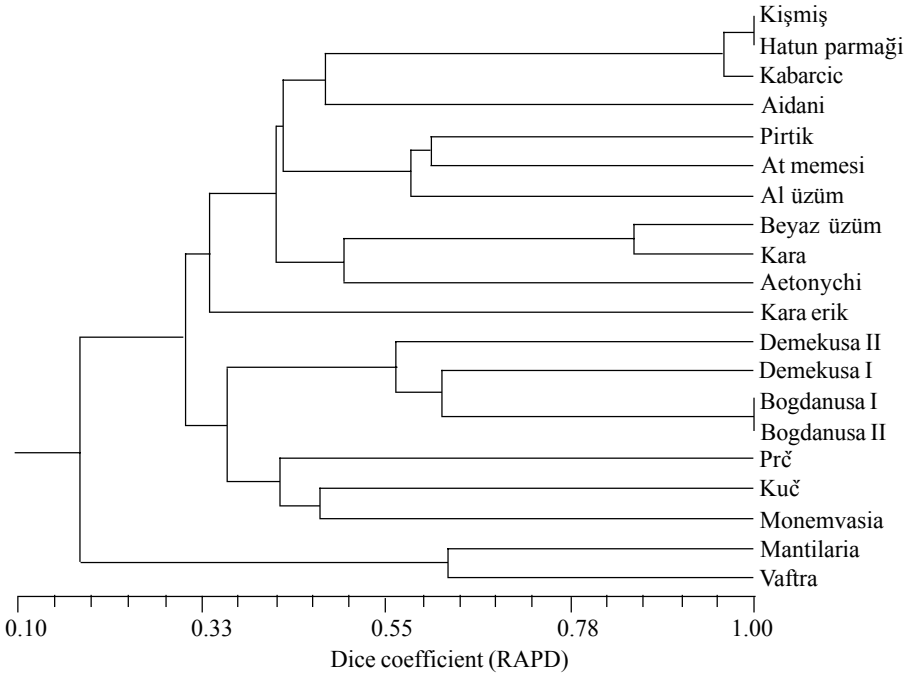


Fig. 2: Dendogram based on Dice genetic similarities among 6 Croatian (HR), 5 Greek (GR) and 9 Turkish (TR) grapevine cultivars, computed from SSR data generated by 8 primers.

T a b l e 3

AMOVA analysis for the partitioning of molecular variation among and within sets of grapevine cultivars from Croatia, Greece and Turkey

| Source of variation | *df* | Variance components | % Total variance | Φ-Statistics | *p-value* |
|---|---|---|---|---|---|
| Among countries | 2 | 2.943 | 17.93 | 0.179 | <0.001 |
| Within countries | 17 | 13.471 | 82.07 | | |

T a b l e 4

$\Phi_{st}$ distance values among sets of cultivars from three countries (lower matrix diagonal), and corresponding *p* values (upper matrix diagonal)

| Country | Turkey | Croatia | Greece |
|---|---|---|---|
| Turkey | | 0.000 | 0.000 |
| Croatia | 0.231 | | 0.078 |
| Greece | 0.180 | 0.094 | |

Hvar was colonized by ancient Greeks. Our investigation did not show direct evidence of any introduction due to the fact that there was no synonym among the analyzed samples. Although the primary aim of this work was to analyze the relationships between three populations of the geographically separated cultivars, some interesting results showed up after further data analysis within the national populations. The 5 Greek samples used in this work were compared on 3 common SSR loci with the SSR data from the Greek Vitis Database, www.biology.uoc.gr/gvd (LEFORT and ROUBELAKIS-ANGELAKIS 2000). Aetonychi and Aidani from our study do not match with those refered in the Greek Vitis Database. Cv. Aetonychi is mentioned to be a very old variety mentioned by Columelle and Plinus in the 1st century A.D. It also has plenty of synonyms and very likely homonyms. This variety was described and genotyped in the Greek Vitis Database but Paros was not referred to as the geographic area for this cultivar. Our sample of Aetonychi, which is probably a homonym, was taken from the village of Paroikia, and has the following properties: black berry, rather late ripening, aromatic, not very high quality, round berry with elongated tip, loose bunch.

Cv. Aidani from this study is probably another homonym because all samples of Aidani in the Greek Vitis Database are not genotyped yet (collection no. 8, originating from Santorini). The other 3 Greek samples match the database on 3 loci analyzed in this study. Although the comparison was made on 3 loci only, it is very likely that these three samples belong to the labeled cultivar names (Mantilaria, Monemvasia and Vaftra). Among the Croatian samples, it seems that the Bogdanuša type I and the Bogdanuša type II

are the clones of the same cultivar. They shared the same SSR profile but revealed polymorphism with two RAPD primers. The morphological differences support this hypothesis. The Drnekuša type I and the Drnekuša type II are quite different cultivars (different genotypes on 7 out of 8 loci) but share alleles on all analyzed microsatellite loci, indicating that they may be closely related. The RAPD results also support this hypothesis (Fig. 1).

Among the Turkish samples Kişmiş and Hatun parmağı have an identical SSR profile, while Kabarcik is almost identical. The difference is only on the VVMD5 locus where the allele 246 was detected in Hatun parmağı and in Kişmiş but was very faint (not scored) in Kabarcik. The RAPD profiles also show high relatedness between those samples but reject identical genotypes. A certain morphological similarity could be observed between these three genotypes of the table grape. At any rate, it is certain that there are three different but closely related genotypes, sampled from the same location (the village of Olur) that might be the clones of the same stock population derived a long time ago. It will be necessary to conduct more extensive ampelographic and DNA analysis to examine this hypothesis.

The cultivars At memesi and Al üzüm also seem to be related. They share alleles on 7 SSR loci out of 8, and the RAPD profiles support their relatedness. They are probably cultivars originating from the same gene pool. They are taken from the same location, and are considered to be autochthonous to that region.

A strong relatedness is found in Beyaz üzüm and Kara. These two cultivars have identical genotypes on 6 out of 8 SSR loci and share alleles on remaining two. The RAPD profiles confirm their relatedness. The morphological differences are obvious, Beyaz üzüm ("white grape") has white berries and Kara ("black") black ones. The leaf morphology differs too. One interesting hypothesis could be that Kara is the progeny of a self-fertilized Beyaz üzüm. This is supported by the SSR results where Kara is a homozygote on VVMD27 and VVMD28 loci, sharing the same alleles with Beyaz üzüm, which is a heterozygote on these loci (see Tab. 2). However, it would be necessary to carry out genotyping on more loci to test this hypothesis.

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# Erratum

In the paper

**Genetic relationships among grapevine cultivars native to Croatia, Greece and Turkey**

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the sampling locations of cultivars Al üzüm and Karaerik in Table 1 have been confused. Al üzüm has been sampled at Olur, whereas Karaerik has been sampled at Erzincan.

The participating institutes have been specified in the title, but author's names haven' t been assigned to their institute. S. ERCISLI is staff of the Faculty of Agriculture, Ataturk University, Erzurum, Turkey. All other authors are members of the Faculty of Agriculture, University of Zagreb, Croatia.

The publishers apologize for this error.