Vitis 41 (3), 159–160 (2002)

Research Note

Molecular discrimination and identification of some Turkish grape cultivars (*Vitis vinifera* L.) by RAPD markers

A. Ergül, B. Marasali and Y. S. Ağaoğlu

Summary: The RAPD-based genetic relationships among 17 indigenous grape varieties (*Vitis vinifera* L.) of Turkey were compared using 22 decamer primers. The genetic relationships among the cultivars concluded from this research are basically related to the origin of the cultivars.

K e y w o r d s : genetic variation, RAPD, Turkey, indigenous grape cultivars.

Introduction: In Turkey with its large grape germplasm, so far, approximately 1200 cultivars, including synonymous cultivars, have been transferred from the different ecological zones of the country to the National Germplasm Repository Vineyard (Çelik *et al.* 2000). In many cases misidentification may have taken place since the conventional criteria of ampelography have prevented inferring the true-to-name grapevine germplasm potential of Turkey. Isoenzyme diversity revealed little genetic variation within the 50 indigenous grape varieties and wild *Vitis sylvestris* types. (Ağaoğlu *et al.* 1995, 1998, 1999; Söylemezoğlu *et al.* 1998, 2001). The study presented was undertaken to determine the levels of RAPD variations within 17 grape varieties which were identified neither by ampelography nor by isoenzyme diversity.

Material and Methods: RAPD conditions: DNA was extracted following the procedure given by Lodhi *et al.* (1994). Amplification was performed in a 25 µl reaction volume containing 200 ng genomic DNA, 2.5 µl 10 x reaction buffer, 3.5 µl of 25 mM $MgCl_2$, 2 µl of 2.5 mM dNTPs, 200 ng primer and 0.5 unit Taq polymerase (Promega). The PCR programme was started with an initial cycle of 94 °C for 5 min and followed by 35 cycles of 30 s at 94 °C, 1 min at 35 °C and 1 min 45 s at 72 °C. Finally extention was performed at 72 °C for 8 min.

Data analysis: Genetic relations between cultivars were determined with respect to the similarity index method (Sokal and Sneath 1963) and a dendrogram was generated by the NTSYS (1.8) computer programme (Rohlf 1990).

Results and Discussion: Out of the 22 decamer primers tested, a total of 179 bands were amplified and 110 of them were polymorphic. The size of the amplified fragments ranged between 200 and 1800 bp. Primers BC 340, BC 374, F 20, OPA 2, OPA 18, P123, P166 and P 394 generated more than 70 % polymorphic bands, while OPA 1, OPA 3, F 12, K 5, K 8, OD 8, P 232, P 402, P 437, P 443 and S 34 primers displayed 50 % or less polymorphism (Figure).

Two major groups were determined from the cluster analysis. The larger cluster consisted of 14 grape cultivars while the smaller one had three. The 17 grape varieties were separated into 4 groups on the basis of genetic variability and regional divergence. Although the cultivars in group 1 (Hafizali, Razaki, Müşküle, Kadin parmağı) have been widely planted in different parts of Turkey, their origin are the Marmara and Aegean regions. In this group, the Hafizali and Razaki showed a high genetic similarity (0.836). Among the paired distances of the cultivars two close values were considerably high. One belonged to the similarity between Hasandede and Narince with 0.851, which are important wine grapes of Central Anatolia. This close relationship let us to classify them in group 2. The other very close and high relationship appeared between Kozak beyazi and Kozak siyahi (0.849), which are local varieties of Kozak in the Marmara-Aegean region. This relationship was assigned to group 4.

Group 3 consists of two cultivars (Tahannebi and Hönüsü) with an 0.814 similarity ratio. They have been grown in Southeastern Anatolia for many years.

The Bozcaada Çavuşu and Amasya cultivars clustered apart from the others in the dendrogram and could not be associated with any group. This result was expected because the cultivars exhibit much more diversity within the varieties, which are grown in sections of different geographic

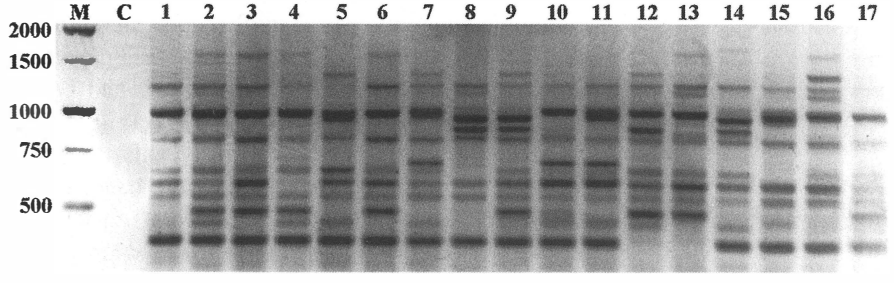

Figure: RAPD patterns obtained with primer P123 (gggATTCgAC), M: Molecular weight marker (bp), C: Negative control, Cultivars (1-17: Bozcaada Çavuşu, Hafizali, Kadin parmağı, Kozak beyazi, Müşküle, Tahannebi, Hönüsü, Kozak siyahi, Emir, Hasandede, Narince, Boğazkere, Öküzgözü, Papaz karasi, Besni, Amasya, Razaki).

Correspondence to: Prof. Dr. Y. S. Ağaoğlu, Department of Horticulture, Faculty of Agriculture, University of Ankara, 06110 Diskapi-Ankara, Turkey. Fax: +90-312-3179119. E-mail: agaoglu@ada.net.tr

regions of Turkey. Boğazkere, Öküzgözü and Besni represented an unexpected discrepancy, despite the fact that they are the local varieties of a specific area between Southeastern and Eastern Anatolia.

Thus, the locations of the 7 other cultivars (Bozcaada Çavuşu, Öküzgözü, Papaz karasi, Boğazkere, Besni, Amasya and Emir) in the dendrogram were not found to be helpful in revealing the genetic relationships among the cultivars and possible sources. These cultivars could not be assigned to any group. For example, from the paired groups constituting a high similarity, the Öküzgözü in Eastern Anatolia and the Papaz karasi in Thrace are varieties which have become localized. Although Emir is connected to the Kozak varieties with a similarity index value of approximately 0.820, it is a variety belonging to the Cappadocia region.

The results of this study indicate that genetic relationships, based on the RAPD identification, partially confirmed relationships derived from ampelographical evaluations. However, regional relationships were more reliable for interpreting the RAPD results.

This work was supported by the Ankara University Research Fund (96-11-01-02). Authors are grateful to Prof. Dr. N. GÖZÜKIRMIZI and Dr. A. ALTINKUT for the use of laboratory equipments in Marmara Research Center (TUBITAK).

AĞAOĞLU, Y. S.; SÖYLEMEZOĞLU, G.; ÇALISKAN, M.; ERGÜL, A.; 1999: Researches on electrophoretic identification of ecotypes of Razaki grape cultivar grown in Turkey (in Turkish with English abstract), 389-394. 3rd Natl. Hortic. Congr., 14-17 September 1999, Ankara, Turkey.

AĞAOĞLU, Y. S.; SÖYLEMEZOĞLU, G.; ERGÜL, A.; ÇALIŞKAN, M.; 1995: Identification of some table and wine grapes grown in Turkey by isozyme banding patterns (in Turkish with English abstract), Vol. 2, 567-571. 2nd Natl. Hortic. Congr., 3-6 October 1995, Adana, Turkey.

AĞAOĞLU, Y. S.; SÖYLEMEZOĞLU, G.; MARASALI, B.; ÇALIŞKAN, M.; ERGÜL, A.; TÜRKBEN, C.; 1998: Identification of some native and foreign grape varieties using isozyme banding patterns by polyacryl-amide gel electrophoresis (in Turkish with English abstract), 145-151. 4th Vitic. Symp., 20-23 October 1998, Yalova, Turkey.

ÇELIK, H.; MARASALI, B.; SÖYLEMEZOĞLU, G.; TANGOLAR, S.; GÜNDÜZ, M.; 2000: Bagcilikta Üretim Hedefleri (Future Prospects in Turkish Viticulture), 645-678. TMMOB Ziraat Mühendisleri Odasi, Türkiye Ziraat Mühendisligi V. Teknik Kongresi, 17-21 Ocak 2000, Ankara. Bildiriler (2).

LODHI, M. A.; YE, G. N.; WEEDEN, N. F.; REISCH B. I.; 1994: A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol. Biol. Reptr. **12**, 6-13.

ROHLF F. J.; 1990: NTSYS-pc Numerical Taxonomy and Multivariate Analysis System. Version 1.8. Applied Biostatistics, New York.

SOKAL, R. R.; SNEATH, P. N. A. 1963: Principles of Numerical Taxonomy. Freeman, San Francisco.

SÖYLEMEZOĞLU, G.; AĞAOĞLU, Y. S.; MARASALI, B.; ERGÜL, A.; ÇALIŞKAN, M.; TÜRKBEN, C.; 1998: Identification of grape varieties by Catechol Oxidize (CO), Peroxides (PER) and Esterase (EST) enzymes extracted from leaves (in Turkish with English abstract), 138-144. 4th Vitic. Symp., 20-23 October 1998, Yalova, Turkey.

SÖYLEMEZOĞLU.; AĞAOĞLU, Y. S.; UZUN, I.; 2001: Ampelographic characteristics and isozymic analysis of *Vitis vinifera* spp. *sylvestris* Gmel. in Southwestern Turkey. Biotechnol. Biotechnol. Eq. **15**, 106-113.