

Research Note

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

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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.

Key words: 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 (ÇELİK *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 MgCl₂, 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 extension 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, Kadın 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

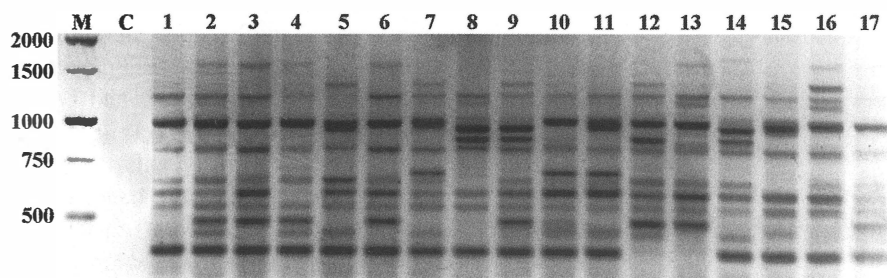


Figure: RAPD patterns obtained with primer P123 (gggATTCgAC), M: Molecular weight marker (bp), C: Negative control, Cultivars (1-17: Bozcaada Çavuşu, Hafizali, Kadın parmağı, Kozak beyazi, Müşküle, Tahannebi, Hönüsü, Kozak siyahi, Emir, Hasandede, Narince, Boğazkere, Öküzgözü, Papaz karasi, Besni, Amasya,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

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.

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