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**“Study of Genetic variation native varieties of sistan grape using  
molecular marker RAPD”**

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**ABSTRACT**

The RAPD data were used to assess genetic similarity among 6 native grape cultivars of sistan. Of the 50 random primers tested on genomic DNA, 21 primer could be selected for genetic analysis. The 6 grape cultivars analyzed with Dice were clustered into four groups.

Grape is an excellent fruit for fresh use or processing into jam, jelly, juicepie, wine. In addition, grapevines can be ornamental and valuable as shade or screen plants in the home landscape when trained on a trellis or arbor. Traditional methods of indentifying grape cultivars have relied on morphological characters whose expression is affected by development and environmental factors. The recently developed techniques, based on the polymerase chain reaction (PCR), offer a new tool for genetic analysis and construction of linkage maps. The random amplified polymorphic DNA (RAPD) technique utilizes arbitrary primers for the amplification of template DNA. This research, in order to study genetic variation of six native Grape genotypes cultivars (Fakhri, Lal, Sangak, Red Yaghooti, White Yaghooti and Cheshm Gavi). We used PCR-based RAPD molecular marker. Six grapes cultivars were collected from sistan grapes in sistan and balochastan province located in east Iran. Genomic DNA was extracted according to the protocol to modified CTAB method. DNA concentrations were determined by photometer.

Genomic DNA was amplified using 50 different RAPD primers. The reaction included 50 mM, 10 mM tris HCL ( PH=8.0) , 0.1% triton x-100 , 2mM mgcl2 , 200 µm each dATP , dCTP,dTTP,dGTP,2unit taq DNA polymerase , 0.4 µm primer and 10 ng genomic DNA , in a final volume of 25 µl. cycling parameters were 40 cycles of 94°C , 30s ; 36 °C , 1min ; and 72 °C , 2 min ;. After the last cycle , the samples were kept at 72 °C for 8 min and then cooled to 4 °C. PCR product were separated by electro phoresis on a 1.4 % agarose gel and visualized by ethidium bromide staining. Each individual was amplified at least twice ; reproducible , polymorphic bands were scored as 1 ( band present ) or 0 ( band absent ). A similarity matrix was constructed with the Dice similarity coefficient .cluster analysis was performed with spss 9.1. While 50 primers were used initially , 21 primers gave optimal amplified DNA , generation a total of 497 bands. Polymorphisms shown among grape cultivars were 220 band. the 6 cultivars were indentified by means of the 21 primers that were selected for their ability to generate unique polymorphic amplification fragments.the mean similarity index for all pairwais comparisons was 53.9 and rang from 41.1( red yaghooti and lal) to 67.2( red yaghooti and white yaghooti) .Similar results were obtained with unweighted pair group method with arithmetic mean(UPGMA). When 6 cultivars were analyzed with UPGMA ( molecular analyst fingerprinting program ) , they were clustered into four groups. The clusters produced by UPGMA are illastrated as a dendrogram (figure 1).

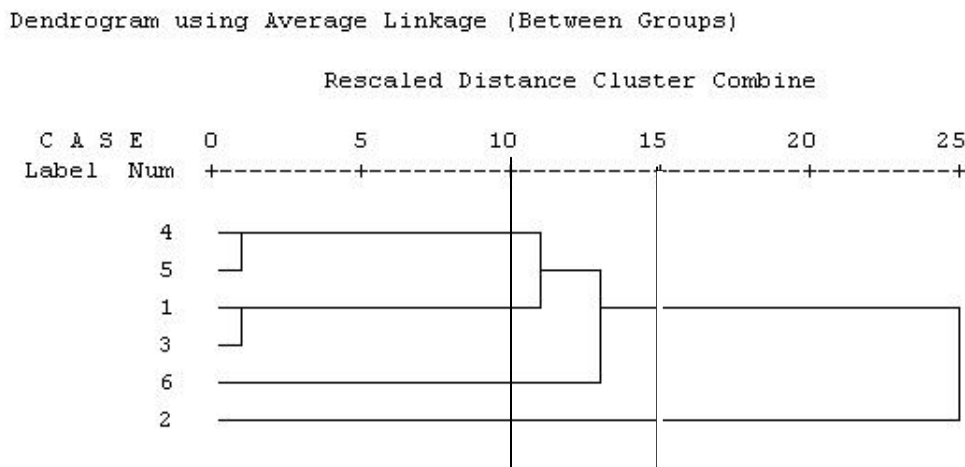


Figure 1:Dendrogram illustrating UPGMA cluster analysis of grape cultivar

**Key word : genetic similarity , random primer , DICE, genetic analysis , similarity matrix**

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