

# Study of polymorphism some native varieties of sistan grape using molecular marker RAPD

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## Abstract

RAPD Marker was used in order to study the genetic variations and assessment of the characters of the grape fruit on the molecular level. For this research 6 genotypes related to the different areas of sistan were assessed by 50 primers. Out of these 50 primers which were tested on the genetic DNA, 21 primers were selected for the genetic analysis. Selected primers produced 497 evident bands. Resulted proliferations lead to the formation of bands ranging in size from 300-3500 base pairs. These bands were grades in the form of (0 & 1) matrix that were in the order of presence of band 1 and the absence of band 0.

Product matrix was analyzed by the method of UPGMA. To draw the dendrogram of the genetic distances of the characters with the help of analysis of clusters were classified in 4 principle groups in which Red Yaghooti and White Yaghooti had the maximum similarity and Red Yaghooti and Lal had the minimum. Considerations multishape bands, efficiency of RAPD marker for the classification of characters has been confirmed in this research.

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

## Introduction

Grapevine (*Vitis vinifera* L.) is an ancient fruit species and its domestication goes back to the 11<sup>th</sup> century BC the long history of grapevine growth has determined a complex picture in which many biotypes or even cultivars are misidentified or called by different names in different areas. This often makes genetic identification difficult. Grapevines are propagated by cuttings and the resulting clones of a given population are genetically identical to each other (except for somatic mutations) and to the mother plant (the original seedling from which cultivars were derived) (8). Traditional methods for the recognition of the characters of grapefruits were on the basis of morphological properties (7) and these properties are also effected by the environmental and growth factors and it results in the decrease in the efficiency of these markers.

Biochemical markers like isozyme were also used for the recognition of the character of the grapefruits . Study of genetics variation on the basis of variation on isozyme was recordable and observable due to less number of polymorphism and recordable difference among them is limited . Therefore by considering the drawbacks of morphological and protein markers . Researchers were persuaded for using the technique of molecular marker which was based on the longitudinal polymorphism of the sectioned pieces of breaking enzymes of DNA.

Their wide applications has been limited due to the following reasons :

- 1- they were expensive
- 2- Their catabolism / breakdown was time taking
- 3- Use of dangerous radioactive material
- 4- Not able to distinguish the characters which are very close to each other .

Therefore we must find the modern markers that will have the maximum efficiency for reforming tasks related to plants .At last in the same case molecular marker based on DNA was produced that showed the wide range of polymorphism on the DNA level and this is the desired method for finding the relative spices which are closed together . With the evolution of PCR techniques new 4 desired techniques based on this technique were spread which can be defined as : Proliferation of the random pieces of DNA or RAPD . Through the benefits of this techniques , RAPD marker has been used on the large scale in population genetics analysis of biological variations and the study of relativity among the spices on the different levels (10).

In the present research , we followed the task which is as follow :Study of genetic variation and or the genetic , polymorphism among the grapefruit culture and consequently the analysis of the data given for the group classification of characters . In this research the application RAPD techniques in Biotechnology laboratory of agriculture department of Zabol university was started in case of grapefruit . **Material**

## **and methods**

### **Plant materials**

Six grapes cultivars (Fakhri,Lal,Sangak,Red Yaghooti, White Yaghooti and Cheshm Gavi) were collected from Sistan grape in Sistan and Balochastan province located in east iran. Till its application it was kept in -70° C and at last analysed through the RAPD method .

## **DNA extraction**

Grind 0.5 g of leaves using mortar and pestle in the presence of liquid nitrogen and their genomic DNA was extracted by protocol Lodhi et al (6) . In this method for taking the polyphenols , PVP and for elimination of the consumed polysaccharides , NaCl was used . After that the DNA plate formed got soluble in TE Buffer . Quantity and quality of genomic DNA was assessed the biophotometry . In this order the basic solutions in which this proportion was between 1.8-2 was selected .After that the working solution or DNA with concentration of 10 nanogram was formed and this concentration of DNA was used in the laboratory .

## **Amplification**

Genomic DNA was amplified using 50 different RAPD primers. The reaction included 50 mM KCl, 10 mM Tris HCl ( pH=8.0 ) , 0.1% Triton X-100 , 2mM MgCl<sub>2</sub> , 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 electrophoresis on a 1.4 % agarose gel and visualized by ethidium bromide staining

## **Analysis**

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 ( Dice , 1945) .cluster analysis was performed with spss 9.1.

## **Results and Discussion**

Observations made in this research showed the suitability of RAPD technique for the ascertainment of the polymorphism among the samples of grapefruit being tested in the laboratory . Formerly this context has been confirmed by the Azeemkhani and Sheeran (2002) , Kigani et al (2001) worked on Brassica and Mohammadyani et al (2002) while worked on pistachio , Lazaro et al(1998) worked on Brassica oleracea , Shah nejat

Bushehri et al (2001) worked on wheat and zlatko et al worked on basii (2002) . On the whole 50 primers were used . 21 primers resulted in the special electrophoretic profiles . Those could be easily classified and has been shown in the figure 1 . In addition , the used primers contained 60-80 % (C+G) and none of the sequences of the primers were the optional or random ones . Collectively 497 bands with 21 primers were graded in order . Bands of every primer were variable between the length of 300-3500 base pairs . More than 2/3<sup>rd</sup> of the bands were bigger than 2027 base pairs and most of them were moderate in size . 88.97 % of the bands were polymorphic (Figure 1) Out of 50 used primers , only 4 of them were not successful for formed by the 2 of them could not graded and 4 of them had the ability to produce the unistructural bands for all of the genotypes . For example B341 primer , at loci of 2027 base pairs and 1584 base pairs produced the evident and clear bonds for all the genotypes.

In this test 11 primers at some of the loci were polymorphous in the form , that bands of similar sizes has been proliferated in all the character . P36 primer produced longest anamorphous and primers B302 . CA2 produced the shortest one contains 564 base pairs .Some of the primers had the ability to make evident bands ranging in size of 200-3530 base pairs having most of the characters . Such as these primers produced the bands with large size with respect to others .

By considering the proliferated DNA , band formed and analysis of data , rate of similarity between the character was calculated . Maximum similarity was between the Red Yaghooti and White Yaghooti which is equal to 0.672 and the minimum similarity was between the Yaghooti Red and Lal colored characters and it is equal to 0.411 .Section of the formed dendogram divided the genotypes in 4 groups . Group 1 contains Yaghooti Red and White Yaghooti genotypes .Group 2 contains Fakhri and Sangak genotypes . Group 3 contains ruby genotype and group 4 contains Chashm Gavi genotype. In reference to figure 2 we can see that in 1<sup>st</sup> group . Firstly Yaghooti Red and White Yaghooti formed the subgroup and like as Fakhri and Sangak genotypes formed the other separate subgroup and genetic distance then Chsham Gavi genotype and at last Lal genotype entered the model .

Results show that genotypes of Lal had the maximum genetic distance with respect to Yaghooti Red and White Yaghooti genotype and Fakhri and Sangak genotype. In the

next step Chashm Gavi genotype had the maximum distance with respect to Yaghooti Red and white genotypes and Fakhri and Sangak genotype .

Fig. 2 ; Dendrogram determining the rate of relativity between the genotype on the basis of coefficient of similarity known as dose with the help of SPSS software , which is divided into 4 groups.

Most important criterias for the selection of marker are : Amount of aviable informations , simple process or work , less expenses , high rate of doing work . In attention to the fact that RAPD marker don't need the use of radioactive elements and having sample sequence in plant genome such as possibility of the use of general primers those can be applied in every species of plant therefore RAPD marker is the suitable marker for the study of genetic variations and relativity rate and also it has the ability to assess the reservoirs of inheritance of the plant species .

In this research 4,5 genotype with respect to genotype and 2 genotype with respect to other genotypes have got more genetic distance therefore with complement studies and better recognition of these genotypes , they can be considered as parents in cross breeds for the production of hybrid varieties . In relation to the variety of hybrid in grape fruit , number of studies have been done . One of the certain way to reach the high heterosis is to use the material that has minimum relativity

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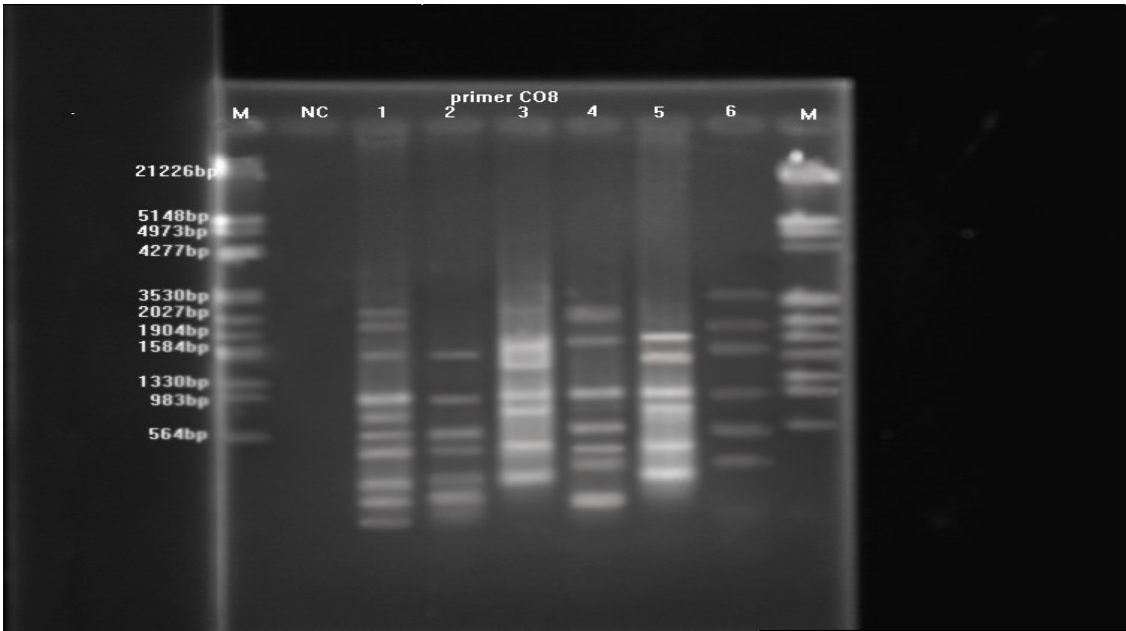


Figure 1. RAPD profiles of grape DNA generated with the 21-mer CO8- (CGCCGCTCCCGATTGGC)

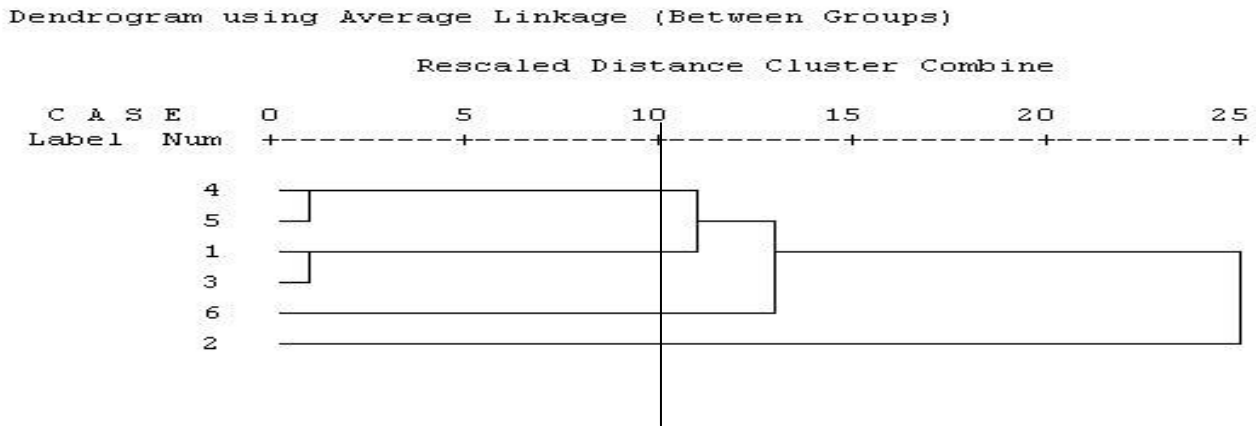


Figure 2 : cluster analysis of 6 cultivars grape