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***Original Contribution***

# THE COMPARISON OF LONG AND SHORT PRIMERS USED FOR RAPD TECHNIQUE IN GRAPE

**N. Mahdi Nezhad[1*], M. Solouki[1], B. Siasar[1]**

[1]Plant Breeding Department, Faculty of Agriculture, Zabol University, Sistan , Iran

**ABSTRACT**

In this research RAPD method was used with the help of individual selective short primers (10 mer)and long primers(15-21 mer).The number of produced polymorphic items ranged in order from 122 and 136 bands for short and long primers. In multiplication reaction , Items in the size area of 1904 to 564 pair base Resulted for short primers and 3530 to 564 pair base for long primers. In this study , by comparing the results gained from techniqe long and short primers in RAPD, the potential value of long primers for the production of polymorphism in grapes was indentified.

**Key words:** random primer, long primer, short primer, RAPD, amplification , polymorphic,grape, PCR

## INTRODUCTION

Random Amplified Polymorphic DNA (RAPD) analysis is a commonly used molecular marker in genetic diversity studies (1). Other related techniques include Arbitrary Primed PCR (AP-PCR) (2) and DNA Amplification Fingerprinting (DAF) (3). These methods differ from RAPDs in primer length, the stringency conditions and the method of separation and detection of the fragments.

The RAPD (random amplified polymorphic DNA) technique has been widely used in plants for the construction of genetic maps in species such as Arabidopsis (4), bananas (5) and slash pine (6), and for genotype identification and taxonomic studies (7, 8). The RAPD reaction is competitive because of the low annealing temperature and short or long primer and template (9). The competitive aspect of RAPD analysis may explain in part why minor changes in almost any aspect of the amplification reaction have been reported to affect the outcome. DNA quality and quantity (1), choice of DNA polymerase (10), mg concentration (11 and 1), choice of thermal cycler (12), primer concentration (1), use of ethidium bromide vs. silver for detection of products (3), and presence of RNA (13)

RAPD markers are detected by the use of short oligonucleotides of arbitrary sequence as primers for the amplification of segments of the target genome. Generally, 10-mer primers with 50-80% G+C content are preferred. As the primer length increases, the genomic target sites decrease because there is less chance of finding perfect or near perfect homologies between the target sites and a longer primer (14) However, complex banding patterns were also generated with primers as short as 5 bases (3). There are few reports on the use of long primers (over 12 bases). The potential value of long primers (15-21 bases) for generating RAPD polymorphisms was investigated in this study.

In this research We compared the use of both short and long primers in RAPD assays of grape in Biotechnology laboratory of agriculture department of Zabol university.

**Plant materials:**

grape cultivars (Fakhri, Laal, Sangak, Red Yaghooti, White Yaghooti and Cheshm Gavi) were collected from the vineyards of Sistan grape in Sistan and Balochastan province located in East Iran. Befor their application they were kept in -70° C and at last analysed through the RAPD method.

*** Correspondence to**: *Nafiseh Mahdi Nezhad ,Department of Plant Breeding , Faculty of Agriculture, Zabol University, Sistan , Iran*
*Tel:00989155426191 Fax:00985422226762*
*Email: nafisehrigi@yahoo.com*

**DNA extraction:**

in the presence of liquid nitrogen 0.5 g of leaves were grinded using mortar and pestle and their genomic DNA was extracted (15) According to this method PVP is added to the buffer to eliminate polyphenols (16), and NaCl for eliminating the excess of polysaccharides (17). After that, DNA is solubilized in TE buffer (Tris 10 mM, EDTA 1 mM pH 8.0). Quantity and quality of genomic DNA was assessed by spectrophotometry. DNA purity was evaluated by obtaining the ratio of the absorbance at 260 nm and 280 nm and only samples with optimal values ranging from 1.8 to 2.0, were used for RAPDs amplification. 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.

**Amplication:**

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, 2 mM MgCl2, 200 µm each dNTPs 2 units Taq DNA polymerase, 0.4 µm primer and 10 ng genomic DNA, in a final volume of 25 µl. 40 cycling parameters consisted of of 94°C, 30 s; 36 °C for short primers and 44-60 °C for long primers, 1 min; and 72 °C, 2 min (Short and long primers were chosen on the basis of what reported by Lodhi et al (18) and Mahmood Solouki (19).

Following PCR 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 in 0.5 x TAE buffer (0.045 M tris, 0.001 M EDTA, acetic acid) and visualized by ethidium bromide staining .

**RESULTS AND DISCUSSION**

While 50 UBC primers were used initially, 21 primers gave optimal amplified DNA, generating a total of 497 bands polymorphism show among grapevine cultivars were 129 bands (%88/97). All 21 primers produced at least one variable locus in all cultivars. Optimal template DNA concentration and primer concentration settled was 10 ng and 0.4 µm, respectively. However, DNA concentration was not affected in these results.

Primers with the lengths of 21, 20, 18, 17, 16, 15 and 10 nucleotides were applied by RAPD marker. Long primers have also been used in genomic fingerprinting. The 16 mer primers (substitute nucleotides with mers; the correct spelling is nucleotides, produced 78 % of polymorphic bands, the 17 and 18-mer primers produced 87%, 20 and 21 mers produced 100% of polymorphic bands. 10 to 15-mer primers produced only 68-70% of polymorphic bands. We observed, however, that long primers yielded more polymorphic bands than the short 10-mer primers, thus increasing the efficiency of RAPD **(Table 1)**.

A typical gel displaying the amplification products generated from grape DNA using long primers is shown in **Fig. 1**. In general, Long primers generated more DNA fragments, a wider range of DNA fragment sizes (typically from 564 to 4277 bp, respect to 564-1904 pb obtained with 10-mer primers) and a greater number of polymorphic fragments than short ones.

We observed that long primers yielded more polymorphic bands than the short 10-mer primers, which is consistent with the observation on poplar (7). Long primers have also been used in genomic fingerprinting (20). However, a few data were presented comparing RAPD results between 10-mer primers and longer primers (19 and 21). It is not clear exactly why the long primers produced more polymorphic bands. It should be pointed out, however, that the long primers used in this study have lower G+C content (from 39% to 55%) compared to commonly used 10-mer primers (more of 50%).

It is not clear in our study whether the increased number of bands produced by long primers was due to lower G+C content, primer length or a combination of both. Future experiments should be designed to determine the effects of primer length and G+C content upon RAPD polymorphisms in a variety of genera.

If the generation of more fragments is due to primer length, one possibility could be that the extra bases at the 5' end, anneal to the templates in a way that either helps the 3' bases anneal to the template to start a new template-primer complex, or stabilizes the unstable existing template-primer complex. Because most of the primers have less than 50% G+C content, intergenic or repetitive DNA regions may be preferentially targeted, which could be useful in mapping telomere and centromere regions that are otherwise not as easily accessible with 10-mer primers 21).

***Table 1.*** *The effect of primer length (10 to 21 bases) on number of RAPD fragments and number of polymorphic fragments per primer*

| Primer Length (Bases) | No. of Primers Tested | Average total no. of Fragments/Primer | Average no. of polymorphic Fragments/Primer |
|---|---|---|---|
| 10 | 7 | 122 | 84 |
| 15 | 2 | 30 | 21 |
| 16 | 2 | 27 | 21 |
| 17 | 2 | 23 | 20 |
| 18 | 2 | 27 | 20 |
| 20 | 4 | 30 | 30 |
| 21 | 2 | 24 | 24 |

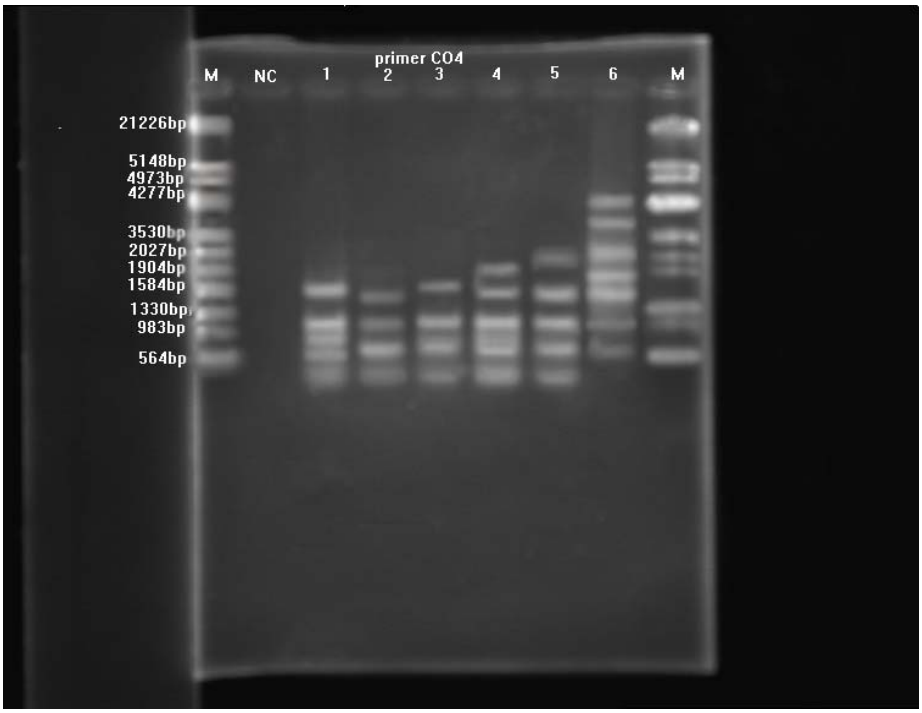


**Figure 1**. RAPD profiles of grape DNA generated with the 20-mer CO4 TGCCTTCCATTCGTAGCCAA

Sequencing of the amplified fragments or probing of the genomic DNA with the amplified fragments would help to determine the nature of amplified products and the mechanism (s) underlying the amplification.

The higher cost for synthesizing long primers can be justified by the greater number of polymorphic bands obtained. Long primers might also be available from colleagues in a large research facility. To generate the same

number of polymorphic bands, reactions with 10-mer primers cost up to three times more in materials and labor as reactions with long primers.

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