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NOTE

RAPD optimization for genetic studies with *Citrulus lanatus* and *Sesamum indicum* genotypes

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ABSTRACT - *The Random Amplified Polymorphic DNA (RAPD) technique is powerful for DNA polymorphism determinations and is widely used in research involving different organisms, but it is known that RAPD can be affected by many factors that may result in false positive bands and non-reproducible assays. In this study, we analyzed the effect of several factors such as DNA template, primer and Taq DNA polymerase concentrations to optimize and standardize the RAPD technique for further genetic studies with* Citrulus lanattus *and* Sesamum indicum *L. The best combination of DNA, Taq DNA polymerase enzyme and primer concentrations in RAPD amplification procedures for sesame and watermelon genotypes was established.*

Key words: DNA amplification; conditions, watermelon, sesame.

INTRODUCTION

The random amplified polymorphic DNA (RAPD) technique, firstly described by Williams et al. (1990), is based on DNA sequences, randomly amplified by arbitrary short single primers using the polymerase chain reaction and tiny DNA quantities. Since no cloning, sequencing or even hybridization is required in this procedure it has become widely-used in the last decade as a very important tool for genetic studies involving different organisms and has replaced more complicated techniques. RAPD enables scientists to compare the polymorphism of random genomic regions with efficiency and little cost instead of polymorphism in one locus only. Garcia et al. (1996) and Lecouls et al. (1999) used the RAPD technique to identify markers linked to nematode resistance genes in *Arachis hypogaea* and *Prunus cerasifera* Ehr, respectively, and Carlton et al. (1995) used it to detect polymorphisms in *Plasmodium*.

In spite of being such a powerful technique to determine DNA polymorphism, used by researchers in numerous studies involving different organisms (Nkongolo et al. 1998, Fraga et al. 2002), it is well-known

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that RAPD can be affected by many factors that can be the responsible for false positive bands and make trials non-reproducible. Specific reaction conditions as well as primer and enzyme concentrations and DNA quality are some of these factors. Pattern reproducibility is frequently named as critical parameter that has to be optimized, as well as the reaction components. Bhat et al. (1999), working with the methodology proposed by Saghai-Maroof et al. (1984) in sesame reported that several modifications in the original procedure are required to obtain reproducible results, apart from the need of optimization.

In this report an optimized RAPD technique was described analyzing critical parameters such as DNA template, primer and *Taq* DNA polymerase concentrations, which are important for reproducible results and necessary for the genetic characterization of watermelon and sesame cultivars. Mainly in the northeastern area of the country these two crops play an increasingly important role for Brazilians farmers. The RAPD characterization of new cultivars and genotypes will certainly be a key tool in breeding programs.

MATERIAL AND METHODS

The *Citrulus lanattus* and *Sesamum indicum* cultivars used in this study were provided by Embrapa Semi Árido and Embrapa Algodão, respectively. DNA of good quality was extracted according to the protocol described by Arriel et al. (2002). The DNA was amplified in a final volume of 25 µL containing 2.5 µL of 10X PCR reaction buffer, 0.2 mM of each dNTP, 1.5 mM of $MgCl_2$ with different DNA template concentrations (10, 20, 40, and 60 ng), 10 MER arbitrary primer in five different concentrations (4, 8, 12, 16, and 24 pmol) and *Taq* polymerase (0.5, 1.0 and 1.5 U), in order to determine their optimal combination. The reaction was performed according to the following amplification profile: an initial denaturation step at 92 °C for 1 min, followed by 42 cycles of 1 min at 94 °C; 1 min at 35 °C and 2 min at 72 °C and 10 min at 72 °C for the final extension.

The analyses were performed by electrophoresis of 15 µL aliquot of the reaction mixture in 1.5% agarose gels stained with ethidium bromide (0.5 mg mL^{-1}) in TBE 1X buffer. Bands were visualized by UV transillumination, and their presence or absence was scored visually. Assays were performed using four replications for each variety plus a negative control to discard any contaminations.

RESULTS AND DISCUSSION

The parameter tested first was the DNA concentration in a range of 10 to 60 ng for both species. It is well known that an adequate amount of DNA is decisive for success with the RAPD procedure. The PCR can work with very few DNA molecules. Using large amounts of DNA would only allow possible contaminants to be transferred from the extraction procedure to the reaction itself. Results showed that 40 ng provided the highest number of bands, as well as good intensity and high repeatability, as shown in Figure 1.

Five different primer concentrations were tested with the DNA concentration determined at 40 ng. Amplification results are shown in Figure 2. As shown for sesame, the best primer concentration was 12. Although at 4, 8, 16 and 24 pmol bands were also recognizable, they were faint and blurred. For watermelon on the other hand, as little as 8 pmol of primer was enough to obtain high quality bands. No significant differences were observed under increasing primer concentrations. Much lower primer concentrations would result in faint bands while higher concentrations would be a waste of the product since the fragment production would not be increased (Ferreira and Grattapaglia 1996).

Once both the DNA and primer concentrations were established, we tested three different enzyme concen-

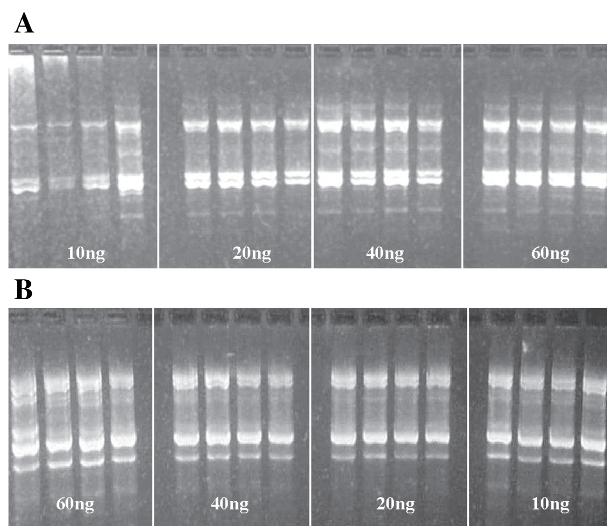

Figure 1. RAPD amplification patterns for different DNA concentrations (10, 20, 40, and 60 ng) obtained with *Citrulus lanattus* (A) and *Sesamum indicum* (B), in 1.5% agarose gel stained with ethidium bromide

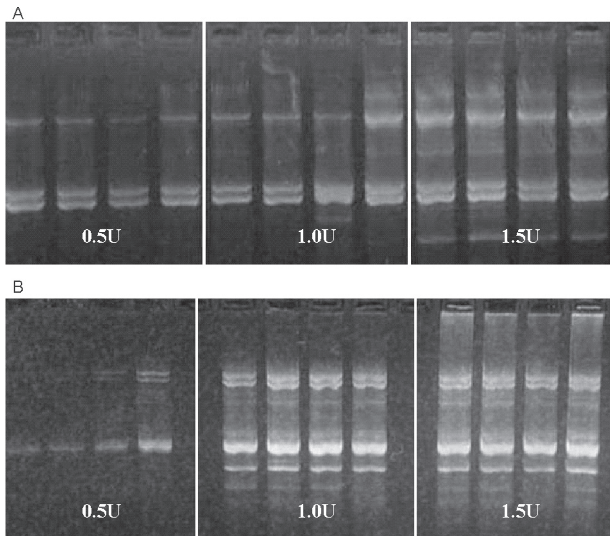

Figure 2. RAPD amplification patterns obtained for *Citrulus lanattus* (A) and *Sesamum indicum* (B) using 40 ng DNA and five different 10 MER primer concentrations in pico moles (4, 8, 12, 16, and 24) in 1.5% agarose gel stained with ethidium bromide, for both tested DNA samples

trations. Results showed that one unit of *Taq* DNA polymerase resulted in the best amplification rate, as shown in Figure 3. Higher concentrations would result in a less specific hybridization pattern. These conditions were optimized by using DNA from one *Citrulus lanattus* and one *Sesamum indicum* genotype. However it has been used with DNA taken from several genotypes, presenting high reproducibility throughout (data not shown) in our RAPD experiments. Our experiments allow the inference that the conditions described above represent the best parameter combination for RAPD for *Citrulus lanattus* and *Sesamum indicum* genotypes.

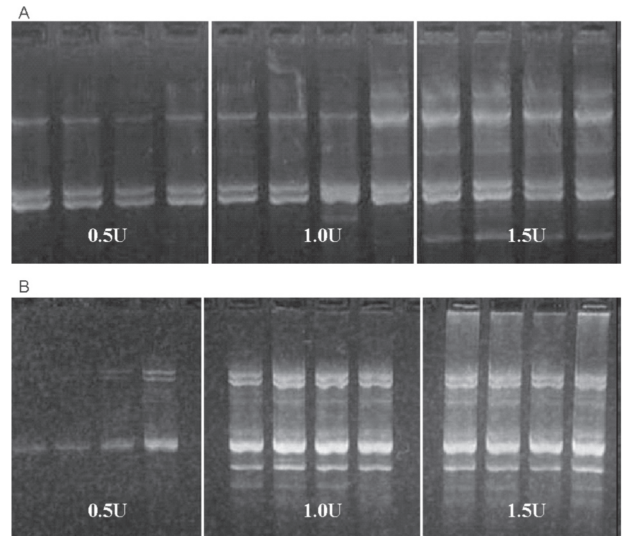

Figure 3. RAPD banding patterns obtained with *Citrulus lanattus* (A) and *Sesamum indicum* (B) in three different *Taq* polymerase enzyme concentrations (0.5, 1.0 and 1.5 U) using 40 ng DNA and 8 and 12 pmol of primer, respectively in 1.5% agarose gel stained with ethidium bromide for both tested DNA samples

Otimização de RAPD para estudos genéticos com genótipos de *Citrulus lanattus* e *Sesamum indicum*

RESUMO - *Apesar do polimorfismo de DNA amplificado ao acaso (RAPD) ser uma técnica poderosa na determinação do polimorfismo do DNA e ser empregado por pesquisadores em muitos estudos relacionados com diferentes organismos, sabe-se que o RAPD é afetado por muitos fatores que podem resultar na presença de bandas falsas, causando a falta de reproducibilidade dos experimentos. Nesse estudo foram analisados os efeitos de diferentes fatores como as concentrações de DNA, dos iniciadores e da Taq DNA polimerase, objetivando otimizar e padronizar a técnica RAPD para os estudos genéticos com* Citrulus lanattus *e* Sesamum indicum*. A melhor combinação dos parâmetros analisados como concentração de DNA, iniciadores e da enzima Taq polimerase para uso em reações de RAPD com as culturas gergelim e melancia é fornecida.*

Palavras-chave: Amplificação de DNA, condições, melancia, gergelim.

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