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A RAPID MINIPREPARATION METHOD FOR DNA EXTRACTION FROM *HEVEA* LEAF TISSUE.

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ABSTRACT

Use of DNA markers in plant breeding is increasingly becoming popular. A basic requirement in using this technique in plant breeding is the availability of a simple inexpensive method for the isolation of plant DNA from large populations. Here we describe a rapid method of DNA isolation from Hevea leaf tissue. DNA yields obtained by this method were comparable with the yields of the other available methods. The quality of the DNA was tested by digesting with several restriction enzymes and was found to be of adequate purity. Southern blot analysis of this DNA showed its suitability for RFLP analysis of Hevea.

Key words: DNA markers, RFLP analysis.

INTRODUCTION

DNA based techniques play an important role in the improvement of plant species. The recognition for the DNA markers as a vital tool in plant breeding came with the development of Restriction Fragment Length Polymorphism (RFLP) based genetic mapping in plants. In addition to the RFLPs, RAPD (Random Amplified Polymorphic DNA) and AFLP (Amplified Fragment Length Polymorphism) are now well accepted for genetic mapping and diagnostic applications. Simple Sequence Repeats (SSR or micro satellites) were recently shown to be abundant and highly variable in plant genomes and efforts are under way to develop SSR based maps of several crop plants (Rafalski *et al.*,1994).

A pre-requisite for successful use of the DNA based technology in plant breeding is the availability of a simple reliable method for the purification of nucleic acids (DNA or RNA) from plants and plant tissue culture material.

Three major criteria should be met by the nucleic acids isolated from plants by such a method (Taylor *et al.*, 1982),

1. The DNA must be pure enough to be cut by restriction enzymes.
2. The DNA must be intact enough to give accurate and reproducible migration patterns following gel electrophoresis.

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3. the yield of DNA must be sufficient so that a reasonable amount of tissue can be used.

DNA extraction methods from plant tissue often require the use of large volumes of expensive reagents and high speed centrifuges with the capacity to handle large volumes. These factors make the DNA isolation procedure a difficult task in an average plant breeding laboratory.

Here we describe a simple, quick and relatively inexpensive minipreparation method of plant DNA isolation from *Hevea* leaf tissue which is as simple as minipreparation methods of plasmid DNA extraction.

MATERIALS AND METHODS

Reagents

Extraction Buffer : 100mM Tris/HCl pH 7.
50mM EDTA
2% N.Lauroylsarcosine

RNAase A : 10μg/μl

Phenol : equilibrated with 20mM EDTA
10mM $MgSO_4$
10mM Tris
0.1%SDS

Phenol/Chloroform :Phenol: Chloroform (1:1)

Chisam :Chloroform: Isoamyl alcohol(24:1)

TE Buffer :10mM Tris
1mM EDTA, pH 8.00

Restriction enzymes : *Eco*R I, *Sau*3A I, *Msp* I, *Hha* I, *Alu* I, *Bgl* II, *Hinc* II, *Pvu* II.

Method

Leaves of *Hevea brasiliensis* cultivars were collected from the RRISL budwood nurseries of the department of genetics and plant breeding, quickly frozen in liquid nitrogen and stored at -70^0 C.

Cell disruption and homogenization

Frozen tissue (400 mg) was ground to a fine powder using liquid nitrogen in a pre-cooled mortar. The powder was transferred to a 2 ml centrifuge tube containing 1.5 ml of extraction buffer and 1μl of RNAase A was added. The contents were then mixed thoroughly and incubated at room temperature with intermittent shaking for one hour, followed by a centrifugation step at 12000 rpm for 10 minutes in a microfuge. The supernatant was then transferred into a new 2 ml centrifuge tube.

Phenol Extractions

The supernatant was extracted with an equal volume of phenol (usually 1 ml). The aqueous phase obtained was further purified by repeating the extractions with equal volumes of phenol/chloroform and Chisam respectively.

Recovery and Purification of DNA

DNA was precipitated at room temperature by adding 75μl of 3M sodium acetate and 500μl of isopropanol to the supernatant obtained from the above step. After centrifuging for five minutes in a microfuge the DNA pellet was recovered, washed twice with 70% ethanol, air dried and resuspended in 150μl of TE.

RESULTS AND DISCUSSION

Table 1 shows the amount of DNA recovered from 400mg of leaf tissue from different *Hevea brasiliensis* cultivars as quantitated by spectrophotometer readings. An average yield of 120μg DNA per gram of fresh leaf tissue could be obtained using the method described. The amount of DNA obtained by this method is comparable with the yields of DNA obtained by other minipreparation methods of plant DNA extraction. For example a widely used method described by Dellaporta *et al.*(1985) reported DNA yields ranging from 50-100μg per gram of leaf tissue from different plant species. But in this method it was necessary to use volumes up to 30 ml and a high speed centrifuge which has the capacity to handle large centrifuge tubes. The amount and the quality of DNA isolated by this procedure was tested by agarose gel electrophoresis using 2μl of DNA from each sample. The migration pattern of the samples (Figure 1) showed that the DNA was of high molecular weight, intact and acceptably concentrated. The DNA obtained from different samples from the same cultivar by repeating the procedure showed a remarkable uniformity (Figure 2).

The suitability of the DNA obtained by this method for restriction analysis was tested by digesting 2.5μg of DNA with several endonucleases. The results showed that the DNA was pure enough to serve as a substrate for all the enzymes used in this study (Figure 3). Southern hybridisation of a genomic blot derived by above restriction digestions (Figure 4) showed that the DNA is suitable for further manipulations such as for RFLPs. We find the method described here for plant DNA extraction is very rapid and efficient. One person can handle up to 18 samples a day. This method circumvents several difficulties encountered by an average plant breeder. Firstly, the minute amount of tissue needed allows the breeder to analyse his material at seedling stage and also overcomes the problem of lack of space for storage of fresh tissue when DNA has to be extracted from large populations. Secondly, this method eliminates the need for a high speed centrifuge. Instead, the whole extraction procedure can be done using only a microfuge. Use of a simple centrifugation method and smaller volumes of the reagents, gives this method the simplicity and the rapidity found in minipreparation methods of plasmid DNA extraction. Further, the preliminary results obtained from different plant species (Figure 5) suggest that this method can be developed for the

extraction of DNA from a variety of plant species.

Table 1. *Yield of DNA (μg) obtained per 400 mg of fresh leaf tissue.*

Sample	Yield Of DNA (μg)
RRIC 100	75.00
RRIC 110	60.00
RRIC 121	43.87
RRIC 130	32.32
RRIC 133	82.62
PB 28/59	16.65
PB 235	25.35
PB 260	62.85
36-471	49.20
42-559	45.07
BPM-24	52.35

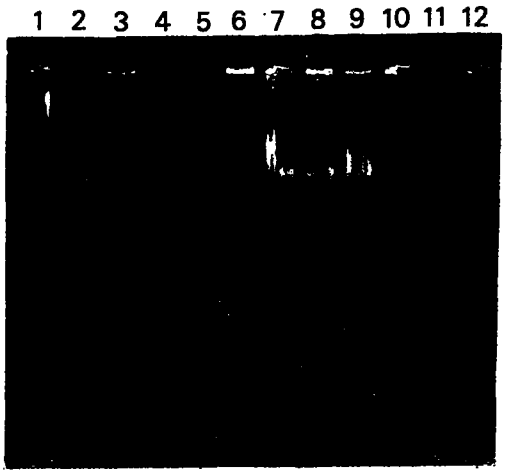

Fig. 1. DNA (2μl) isolated from different *Hevea* clones, run on a 0.8% agarose gel. lanes 1-12, represent Lambda DNA 250ng, RRIC 100, RRIC 102, RRIC 110, RRIC 121, RRIC 133, PB 28/59, PB 260, 36- 471, 42-559, BPM 24 and PB 235 respectively.

Minipreparation method for DNA extraction

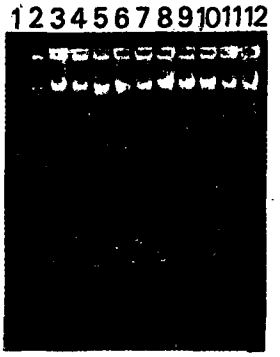

Fig. 2. DNA (2μl) isolated from different samples of a clone. Lanes 1 & 2: RRIC 130. Lanes 3, 4, 5, 6 & 7 : RRIC 133. Lanes 8, 9, 10, 11 & 12 : PB 28/59.

Fig. 3a. 2.5μg of DNA from different clones were digested with 20 units of *Eco*R I enzyme for 3 hours and were separated on a 0.8% agarose gel. Lanes 1-10, Lambda DNA, RRIC 100, RRIC 100, RRIC 102, RRIC 121, RRIC 130, RRIC 133, PB 28/59, PB 217 and PB 260.

Fig. 3b. 2.5μg DNA digested with 12 units of *Sau*3A I for 3 hours and separated on a 0.8% agarose gel. Lanes 1-8, RRIC 100, RRIC 100, RRIC 102, RRIC 110, RRIC 121, RRIC 130, RRIC 133 & PB 28/59.

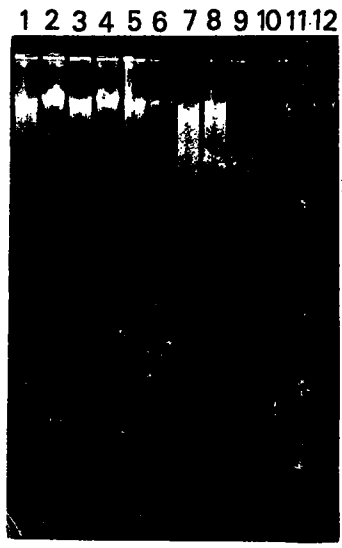

Fig. 3c. Lanes 1, 3, 5, 7, 9 and 11 : 2.5μg of DNA from clone RRIC 102 digested with *Msp* I, *Hha* I, *Alu* I, *Bgl* II, *Hinc* II and *Pvu* II respectively. Lanes 2, 4, 6, 8, 10 and 12 : 2.5 μg of DNA from clone PB 28/59 digested with *Msp* I, *Hha* I, *Alu* I, *Bgl* II, *Hinc* II and *Pvu* II respectively.

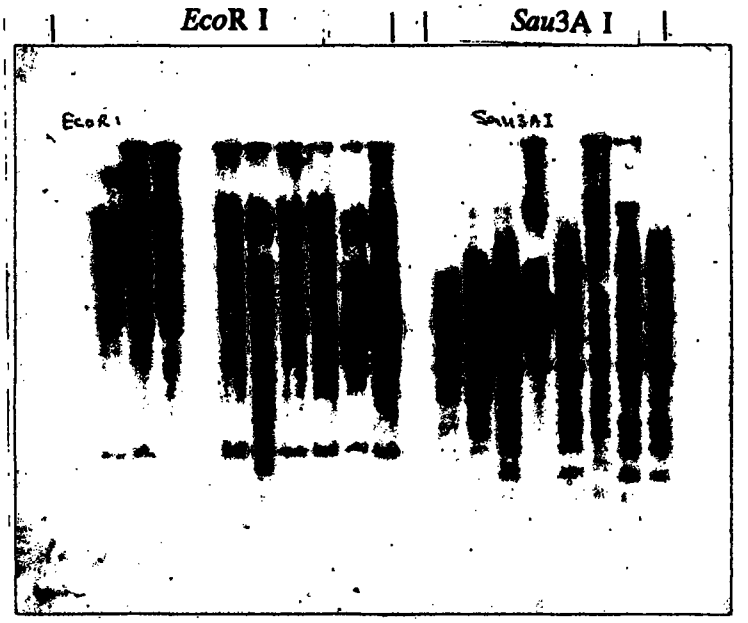

Fig. 4. Southern analysis of DNA from different *Hevea* genotypes digested with *Eco*R I and Sau3A I and probed with a repetitive DNA sequence. Filters were hybridized overnight at 42^{0}C using 50% Formamide, washed in 2xSSC at 65^{0}C for 3 hours and exposed to a X-ray film for 12 hours.

Fig. 5. DNA (2μl) extracted from different plant species.
Lane 1 : *Hevea spruceana*
Lane 2 : *Oryza sativa*

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