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A NOTE ON ISOLATION OF DNA FROM BARK TISSUES OF MATURE RUBBER TREES (*HEVEA BRASILIENSIS*)

The isolation of good quality DNA in sufficient quantity is a pre-requisite to various studies in molecular biology. Young tissues, most often leaves, are the best materials for DNA isolation. Young tissues contain fairly low concentrations of phenolic compounds and are relatively free of starch and other polysaccharides when compared to mature tissues. These compounds are known to inhibit the Taq polymerase enzyme and thus make it difficult to amplify DNA in the polymerase chain reaction (Fang *et al.*, 1992).

A few protocols are available for the isolation and amplification of DNA from leaves of *Hevea brasiliensis* (Herath *et al.*, 1996; Varghese *et al.*, 1997). While total RNA has been successfully extracted from *Hevea* bark tissues (Venkatachalam *et al.*, 1999), no report is available on the isolation of DNA for PCR amplification from the bark tissues of *Hevea*. Isolation of amplifiable DNA from woody tissues can be very difficult due to the presence of several interfering substances such as tannins, polysaccharides, polyphenols, lignins, *etc.* (Murray and Thompson, 1980). In order to study the rootstock-scion interactions at molecular level, DNA has to be isolated from mature bark tissues of *Hevea*.

In the present investigation, a protocol for the isolation and amplification of DNA from bark tissues of the rootstock and scion portions of mature rubber trees is reported.

Bark samples from the rootstock and scion (clone GT 1) were collected from the Central Experiment Station of the Rubber Research Institute of India at Chethackal and transported to the laboratory on ice. The samples were then washed with sterile water and dried with filter paper. About 0.5 g of soft bark tissues, wrapped in aluminium foil were frozen in liquid nitrogen and kept at –60°C until use. A method described by Porebski *et al.* (1997) for extraction of DNA from mature strawberry leaves that contain high concentrations of polyphenols and polysaccharides was used with modifications. The bark samples were ground to a fine powder in a pre-chilled mortar using liquid nitrogen. The powder was scooped into an Oakridge centrifuge tube and mixed with 5 ml pre-warmed (60°C) extraction buffer (100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, pH 8.0) containing 0.3% β-mercaptoethanol, which was added to the buffer immediately before use. Following this, 50 mg polyvinyl-pyrrolidone (Sigma) was added. After mixing the contents, the tubes were incubated in a water bath at 60°C for 30 min. Chloroform : isoamyl alcohol (24:1 v/v, 6 ml) was added to the tube when the contents of the tube attained room temperature. After

mixing thoroughly, the tubes were spun at 3000 rpm for 20 min at room temperature. The top aqueous phase was transferred into a fresh centrifuge tube and the extraction was repeated using chloroform : isoamyl alcohol. Sodium chloride (5 M, 0.5 volume of the final aqueous solution recovered) and ice cold ethanol (95%) were added to the extract and mixed well. The tubes were kept at –20°C for 10 min after mixing by inversion and was stored at 4°C overnight. The tubes were spun at 3000 rpm for 6 min and the pellet was washed with ice cold 70% (v/v) ethanol. The DNA pellet was dried at room temperature and dissolved in 300 ml of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

The DNA solution was transferred to a 1.5 ml Eppendorf tube and 3 µl of RNase A (10 mg/ml) was added. The tubes were incubated in a water bath at 37°C for 1 h. Proteinase K (3 µl of 1 mg/ml Sigma) was added to this and the incubation was continued at 37°C for 30 min. Neutral phenol (300 µl) was added to each Eppendorf tube, vortexed briefly and centrifuged at 12000 rpm for 10 min. The upper layer was collected in fresh 1.5 ml tubes and the DNA was precipitated by adding 1/10 volume of 2 M sodium acetate and 2 volumes of absolute ethanol and kept at –80°C overnight. The tubes were centrifuged at 12000 rpm for 10 min and the DNA pellet was washed with ice cold 70 per cent v/v ethanol. The pellet was dried at room temperature and then dissolved in 200 µl TE.

Quantification of purified DNA was done through spectrophotometric measurement of UV absorbance at 260 nm. Three micrograms of the sample were digested with *Eco*RI enzyme (15 units) at 37°C for 1 h. PCR amplifications were carried out using four Operon arbitrary decamer primers (OPA-10, OPD-08, OPE-01 and OPB-15). PCR was performed in a total reaction volume of 25 µl and contained 10 picomoles primer and 50 ng template DNA, 1X polymerase buffer (Promega, USA), 2 mM $MgCl_2$, 0.2 mM dNTPs and 0.7 unit of Taq DNA polymerase (Promega, USA). Samples were initially heated to 94°C for 3 min followed by 40 cycles of denaturation at 94°C for 30 sec, annealing at 36°C for 1 min, polymerisation at 72°C for 2 min and a final extension at 72°C for 7 min. PCR amplicons were then run in a 1.5% agarose gel, stained with ethidium bromide and visualized using a UV light source.

The average yield of DNA ranged from 50 to 100 mg/g of bark tissues indicating usefulness of the protocol adopted. These DNA samples had A_{260}/A_{280} ratios ranging from 1.6 to 1.8 and were completely restrictable using *Eco*RI (Fig. 1). All the DNA samples were amplified by PCR and yielded

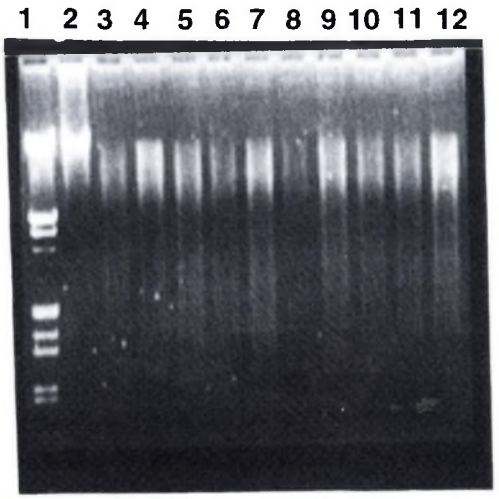
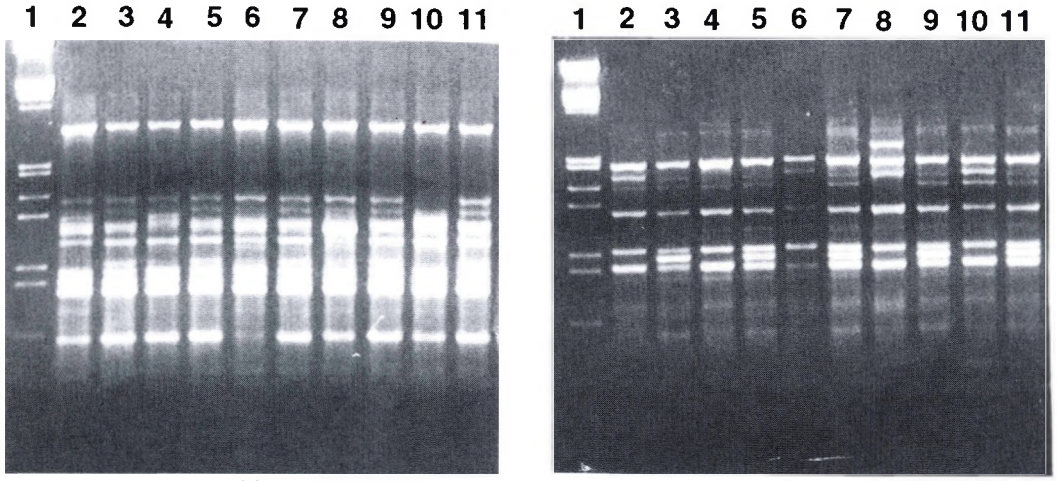


Fig. 1. Restriction digestion of bark DNA samples with *Eco* R1 Lane 1 : DNA marker, 2 : uncut DNA, 3-12 : DNA samples digested with *Eco* RI.



(a) (b)

Fig. 2. RAPD profiles of the bark DNA samples of *Hevea* generated with the primer OPD-08 (a) and OPA-10 (b). Lane 1 : DNA marker, lanes 2-11 : bark samples from stock and scion (lanes at even number positions are stock samples and at odd number positions are scion samples; with every two adjacent lanes starting from lane 2 representing the same tree).

reproducible RAPD profiles with the four primers tested. The RAPD profiles of the bark DNA using the primers OPD-08 and OPA-10 are given in Figure 2. As expected, the DNA profile of the scion tissues were identical (lanes 3, 5, 7, 9 & 11) because they were all from different trees of the same clone, GT1. The DNA profiles from the rootstocks were different, confirming their genetic heterogeneity (lanes 2, 4, 6, 8 & 10). This protocol is a simple and reliable procedure for the isolation of amplifiable DNA from the bark tissues of *Hevea*.

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