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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