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A SIMPLE AND EFFICIENT METHOD FOR THE ISOLATION OF TOTAL RNA AND mRNA FROM MATURE LEAVES OF *HEVEA BRASILIENSIS*

Efficient extraction of high quality RNA from plant tissues is an important step required in most molecular biology work such as northern hybridization analysis, cDNA library construction, *in vitro* translation etc. Preparation of high quality RNA from *Hevea* leaves confronts with difficulties due to the presence of high levels of phenolics, carbohydrates or other compounds that bind and/or co-precipitate with the RNA (Venkatachalam *et al.*, 1999).

For a rain-fed tree crop like *Hevea* drought is a major limiting factor for growth and productivity. Several physiological parameters are adversely affected by abiotic stress in plants. Plants utilize a number of protective mechanisms against these stresses, which maintains normal cellular metabolism and prevents damage to the structure and function of cellular components (Ingram and Bartels, 1996). One fundamental metabolic alteration is the accumulation of stress-induced gene products (Pelah *et al.*, 1997). Some *Hevea* clones are tolerant while others are susceptible to drought stress. It is possible to identify the genes responsible for drought tolerance by molecular biology techniques, which are becoming increasingly relevant in stress physiology research. These genes can be isolated and used as probes for the identification of stress tolerant genotypes among the wild *Hevea* germplasm collection and the hybrid progenies that are generated annually through hybridization programmes. It is also possible to induce over expression of these genes in high yielders so that they withstand the extreme environmental stresses and continue to be high yielders. For such studies a simple and reliable method for the isolation of good quality RNA from *Hevea* tissues in sufficient quantities is a pre-requisite.

Methods for RNA isolation and cDNA library construction in *Hevea* have been reported in the past (Kush *et al.*, 1990; Goyvaerts *et al.*, 1991; Chye *et al.*, 1991; Chye and Cheung, 1995; Venkatachalam *et al.*, 1999). In order to standardize a simple protocol for the isolation of good quality RNA from physiologically mature leaves of *Hevea*, different protocols *viz.*, Goldsbrough *et al.* (1986), Pawlowski *et al.* (1994) and Salzman *et al.* (1999) were tried. It was found that the method as described by Salzman *et al.* (1999) with some modifications gave good quality RNA. In the present study a modified version of this protocol was adopted for the isolation of RNA from mature leaf tissues of *Hevea* that are rich in polysaccharides and polyphenol components.

Hevea plants were grown in the green house by germinating seeds. Physiologically mature leaves from eight-week-old plants were collected in liquid N_2 and kept at –70°C until use. About 0.5 g of leaf tissue was ground in liquid N_2. The powder was scooped into Oakridge tube with 5 ml extraction buffer (4 M guanidine thiocynate, 100 mM Tris HCl, pH 8.0, 25 mM Sodium citrate pH 8.0, 0.5% N – lauryl sarcosine) containing 50 mg PVP-40 and 100 µl β-mercaptoethanol, which were added to the buffer just prior to use. The contents of the tube were mixed by vortexing the tubes for one minute and 5 ml of chloroform : isoamyl alcohol mixture (24:1 v/v) was added to the tube and the vortexing continued for another 10 minutes. The tubes were spun at 12,000 rpm for 15 minutes at 4°C. The top aqueous phase was transferred into a fresh centrifuge tube and the extraction repeated using chloroform : isoamyl alcohol mixture. The top aqueous phase was again trans-