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CELL WALL PROTEINS AND TAPPING PANEL DRYNESS SYNDROME IN RUBBER (*HEVEA BRASILIENSIS*)

High yielding rubber trees often succumb to a physiological disorder commonly termed 'brown bast' (Tapping Panel Dryness - TPD), when the exploitation of latex from rubber trees exceeds their physiological efficiency for latex regeneration. The most common symptoms of this include a phase of excessive late dripping and simultaneous drop in rubber content, besides cracking, thickening and browning of bark (de Fay and Jacob, 1989) and other bark modifications such as soft bark, hyperhydrated bark and necrotic areas in bark. Until recently, a major amount of work with a view to understanding the cause of this syndrome was restricted to latex biochemistry and the general physiology of the tree and a number of postulates were put forward. The present report describes the isolation, preliminary characterisation and an analysis of *in-vitro* role of bark cell wall proteins from healthy and affected plants.

As rubber trees are budgrafted, bark samples from both stock and scion of healthy trees and trees exhibiting varying degrees of TPD were collected and preserved in ice. The outer dry bark was scrapped off and the remaining live bark was collected and used as bark sample. The scrapping in case of normal trees were upto a depth from where latex just began to exude. The same depth was fixed for scrapping off bark from affected trees.

The bark surface was cleaned with distilled water to remove adhering coagulated rubber particles, blotted, dried, weighed and pulverised finely in liquid nitrogen. The method of Tanaka and Uchida (1979) was followed for the extraction of proteins. The bark was extracted (1:5, w/v) with sodium acetate buffer, 50 mM (pH 5.6) containing 300 mM NaCl. The extract was left on an orbital shaker for a minimum of 6 hr to improve solubilization of proteins. Later, it was centrifuged at 27000 g for 20 min. The supernatent was collected and proteins were precipiated with 2.5 vol of chilled acetone for a minimum of 4 hr at 4°C. The precipitate was collected by centrifugation, briefly air dried to remove traces of acetone and resuspended in 50 mM acetate buffer, pH 5.6 (3-4 ml buffer/5 g bark). It was centrifuged at 27000 g for 10 min at 4°C, and proteins in the supernatent were reprecipitated with acetone and the precipitate suspended in 50 mM sodium acetate buffer, pH 5.6 (1:0.1, w/v). The extract was again centrifuged at 27000 g for 10 min at 4°C. The clear supernatent obtained was dialysed extensively against 50 mM acetate buffer, pH 5.6 and used for most subsequent analyses. However, for amino acid composition analysis, the major glycoprotein alone was electroluted from 5 per cent native polyacrylamide gels and used.

The glycoproteins were deglycosylated by the method of Sojar and Bahl (1987) using trifluoromethanesulfonic acid (TFMS). The deglycosylated protein was made free of reagents and low molecu-