Analysis of the protein profiles of healthy and TPD-affected *Hevea brasiliensis* bark tissues

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Abstract

The present study was conducted with the objective to understand the bark protein profiles of healthy and TPD-affected *Hevea*. Soft bark tissues were collected (2 - 3mm thick laticiferous tissue adjacent to the cambium) from both fully dry and healthy trees of clone RRII 105. The tissue was homogenized in borate buffer (pH 9.0) and the total buffer soluble protein was extracted and estimated. The extract was then subjected firstly to 85°C and subsequently 95°C heat treatment for 20 minutes. The heat stable proteins were collected and estimated. The crude extract and heat stable fractions (HSFs) were further analyzed by electrophoretic separation (SDS-PAGE). In addition, the C-serum proteins in healthy and late dripping trees were tested for the presence of stress induced LEA group of proteins.

The buffer soluble protein content did not differ significantly in either the crude extract or HSF at 85°C between TPD-affected and healthy trees. However, in the HSF at 95°C proteins were significantly higher in the healthy trees versus the TPD-affected trees. When shocked at 85°C there was a 25% reduction in the protein content of the crude extract as against 31% in case of TPD-affected bark. These reductions were 51% and 58% at 95°C in the healthy and TPD-affected bark, respectively. These results suggest that the bark proteins of *Hevea* are remarkably tolerant to heat shock.

The protein profile analysis by SDS-PAGE revealed the presence of a protein (approx. 70kDa) in TPD-affected bark in relatively higher quantities which seemed to be relatively heat stable. In addition to this, some new sets of heat stable proteins in the lower molecular weight range (<30kDa) were also noticed in TPD-affected bark. Further investigations on the significance of these proteins are in progress.

A dot-blot analysis using LEA-2 and LEA-3 group polyclonal antibodies revealed the presence of LEA proteins in the C-serum in both healthy and late dripping trees. The LEA and heat stable proteins are known to impart stress tolerance in plants. Hence, their role in TPD is being investigated.

Introduction

In response to various biotic and abiotic stresses, plants have developed different physiological and biochemical strategies to adapt or tolerate stress conditions. The most common strategies are changes in normal metabolic activity, accumulation of low molecular weight protective compounds, sugar alcohols, glycine betaine etc, and, in addition to these,a large set of genes is transcriptionally activated which leads to accumulation of new proteins[1]. It is assumed that the stress induced proteins play a role in imparting tolerance to the cells[2].

Stress induced proteins in plants are of different kinds and in some cases different stresses (such as salinity or drought stress) induce the synthesis of the same set of proteins. One of the common stress induced proteins in plants is LEA (late embryogenesis abundant) protein and it was first characterized in cotton[3]. Based on the similarities in amino acid sequence, six groups of LEA genes have been identified. The majority of LEA gene products are hydrophobic, lack Cys and Trp amino acids, and are located in the cytoplasm. Based on the individual amino acid sequence and predicted protein structure, specific functions have been proposed for each species of LEA[4] such as sequestration of ions, protection of other proteins or membranes, renaturation of unfold proteins etc. It has been hypothesized that based on the correlation of LEA gene expression with physiological and environmental stress, the LEA proteins may play a protective role in plant tissue under various stress conditions[2]. Recently, Jayaprakash et al[5] observed positive correlation between LEA 2 and 3 protein accumulation and the growth rate under abiotic stress in annual crops.

RRDB/Workshop on Tapping Panel Dryness in Hevea brasiliensis. 29–30 April 1997, Hainan, China, pp. 17–20

Tapping panel dryness (TPD) in *Hevea* is considered as a physiological disorder and can be treated as biotic stress. Many kinds of biochemical and physiological responses have been observed in TPD-affected trees of *Hevea*. Recently, KrishnaKumar et al[6] reported some of the common biochemical responses under abiotic and biotic stress (TPD) conditions. However, the information on the stress induced proteins are not available in TPD-affected trees. Studies on stress responsive proteins, such as heat shock, LEA proteins etc, may give some usetul information which will help to understand and characterize this disorder in *Hevea*. Probably high LEA content in the tissue may induce tolerance to this disorder. With this background, experiments were conducted with the objective to study the protein profiles in the bark of normal and TPD-affected *Hevea*. Experiments were also carried out to test the presence of LEA proteins in the latex of *Hevea*.

Materials and methods

Bark sampling and protein extraction

Hevea brasiliensis (Clone RRII 105) trees under regular tapping treatments (S/2 d/3) were selected for collection of bark and latex samples. Normal and TPD-affected trees were marked and monitored regularly. Soft bark tissues (2 - 3mm thick laticiferous tissue adjacent to the cambium) from fully dry and healthy trees were collected. The tissue was powered in liquid nitrogen and stored at -80°C. For total buffer soluble protein extraction, the tissue was homogenized in borate buffer (50mM Sodium borate, 50mM Ascorbic acid, 1% b-mercaptoethanol, lmM PMSF, pH 9.0) and centrifuged at 20,000rpm for 35 minutes at 4°C. The supernatant fraction was used to estimate the total buffer soluble protein by following the modified procedure of Bradford[7].

Heat stable protein separation

The extract was subjected heat treatment firstly at 85°C and then at 95°C for 20 minutes by holding in a water bath. The heat stable proteins were collected by subjecting the extract to centrifugation at 20,000rpm for 20minutes. The supernatant was collected and protein was estimated.

Analysis of protein profiles

The crude extract and heat stable fractions (HSFs) were further analyzed by electrophoretic separation (10% SDS-PAGE). Equal amounts of proteins were subjected to electrophoretic separation and the protein profiles were analyzed by staining the gel in Coomassie brillient blue (CBB) dye binding technique[8].

Analysis of LEA proteins by dot-blot assay

Latex samples from normal and trees showing early TPD symptoms (late drippers) were collected and subjected to centrifugation at 30,000rpm for 30 minutes to collect the C-serum. Proteins were estimated in the serum and a known amount of protein was coated onto a nitrocellulose membrane for dot-blot assay using LEA-2 and LEA-3 groups of antibodies. The antisera were raised again conserved peptide region of LEA-2 (EEKKGIMDKDIKELPG) and LEA-3 (TAQAAKEKEGE) proteins synthesized chemically and were conjugated with a carrier protein BSA. The assay was carried out as described by Tijssen[9] with minor modifications.

Results and discussion

The buffer soluble protein content did not differ significantly either in the crude extract or in HSF at 85°C between TPD-affected and healthy trees (Figures la and b). However,proteins were significantly more in healthy than the TPD-affected trees in the HSF at 95°C (Figure 1c). When shocked at 85°C there was a 25% reduction in the protein content of the crude extract as against 31% in the case of TPD-affected bark. At 95°C these reductions were 51% and 58% in the healthy and TPD-affected bark, respectively. These results suggest

that the bark proteins of *Hevea* are remarkably tolerant to heat shock. Similar kinds of reports are also available in other woody species bark[10].

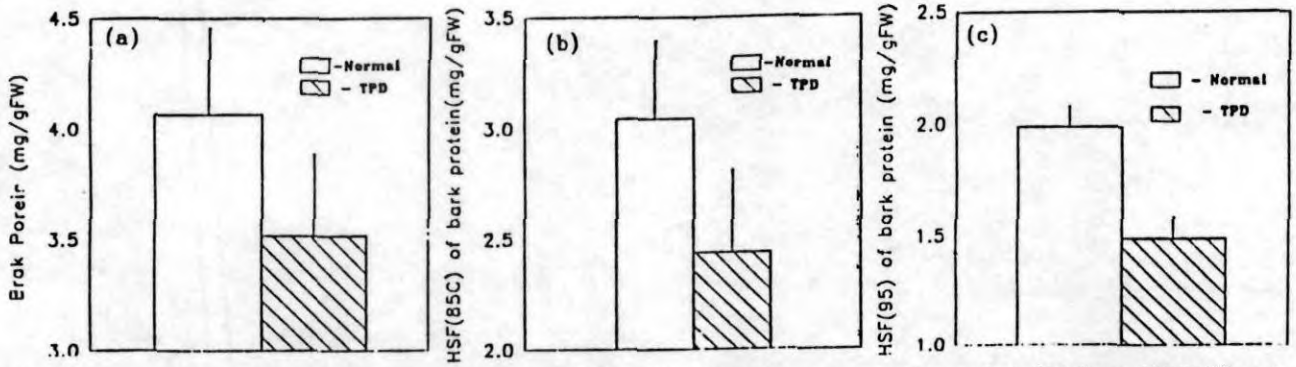

Figure 1 ***Bark protein content in clone RRII 105: a) crdue extract; b) and c) 85°C and 95°C heat stable proteins, respectively.***

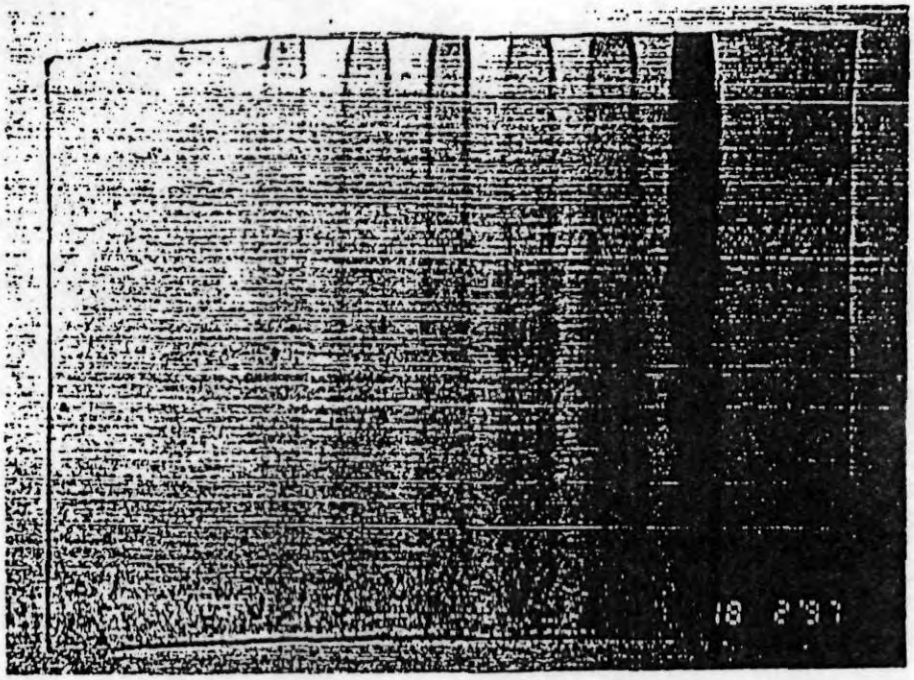

Plate 1 ***SDS-PAGE confirming the presence of one protein (70kDa approx) with a relatively higher content in TPD affected tree bark. Lane 1 - marker proteins (MW 205,000; 116,600; 97,400; 66,000; 45,000 and 25,000). Lanes 2 and 3 are crude extract of normal and TPD-affected bark protein, respectively. Lanes 4 and 5 are 85°C heat stable protein and Lanes 6 and 7 are 95°C heat stable proteins of normal and TPD-affected bark tissue.***

The protein profile analysis by SDS-PAGE revealed the presence of protein (approx. 70kDa) in TPD-affected bark in relatively higher quantity which seems to be relatively heat stable (Plate 1). In addition to this, some new set of heat stable proteins in the lower molecular weight range (<30kDa) were also noticed in TPD-affected bark. Further investigations on the significance of these proteins are in progress.

A dot-blot analysis using LEA-2 and LEA-3 group polyclonal antibodies revealed the presence of LEA proteins in the C-serum in both healthy and late dripping trees. The LEA and heat stable proteins are known to impart stress tolerance in plants[2,5]. Observed positive correlation between LEA 2 and 3 protein accumulation and growth rate under abiotic stress in annual crops indicate the relevance of these proteins in normal plant metabolism. It is considered therefore that TPD is a biotic stress, possibly triggered by different factors such as over-exploitation and hence the role of LEA and stress induced proteins in TPD is being investigated.

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Discussion

Dr Jean-Louis Jacob (CIRAD) stated that sampling techniques are very important: there is a need to detect precisely when and where events occur.

Dr James Jacob (RRII) replied that the trees were 100 per cent fully dry, but without necrosis in the bark below the tapping panel. The samples were taken from a 2 - 3mm thickness of soft bark tissue.

Dr Jean-Louis Jacob (CIRAD) asked whether the bark had been scraped.

Dr James Jacob (RRII) replied that the objectives were to establish whether any heat shock proteins or any LEA group proteins are present as these are known to impart tolerance to stress.