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# DISTRIBUTION OF PROTEIN-STORING CELLS IN THE BARK TISSUE OF *HEVEA BRASILIENSIS* IN RESPONSE TO TAPPING PANEL DRYNESS AND STIMULATION

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Latex from *Hevea brasiliensis* is harvested through controlled wounding (tapping) of the latex vessels present in the bark tissue. The continuous injury on the bark leads to regeneration of the tissue involving various anatomical and biochemical changes in the cells adjacent to the wounded bark. In general, trees of high yielding *Hevea* clones when subjected to over-harvesting latex are susceptible to tapping panel dryness (TPD), resulting into the cessation of latex flow. The actual mechanism involved in the partial or complete cessation of latex flow in the TPD affected trees is still obscure.

The distribution and occurrence of protein-storing cells (PSC) in the secondary phloem (bark) are a common phenomenon which is considered as an end product of various translocation mechanisms taking place in the bark tissue. In healthy trees of rubber, the PSC was distributed in one or two layers surrounding the phloic rays and also adjacent to sieve tubes, where as its density was increased in TPD affected trees (4-5 layers) as well as in TPD affected regions of stimulated trees. The PSC were in close association with the sieve elements which were an important in the movement of photosynthates. Occurrence, structure and distribution of PSC and its functional role in *Hevea* bark tissue with respect to TPD and stimulation were studied and discussed.

**Key words:** *Hevea brasiliensis*, Protein storing cells, Stimulation, Tapping panel dryness.

## INTRODUCTION

*Hevea brasiliensis*, the prime source of natural rubber, is harvested through controlled wounding (tapping) of latex vessels present in the bark tissue (Fig. 1). The continuous injury of the bark leads to a series of anatomical and histochemical changes in the cells adjacent to the wound leading to bark regeneration as the cambium is undisturbed (Thomas *et al*., 1995). In general, trees of high yielding clones of *Hevea* when subjected to over-harvesting latex are susceptible to a disorder termed tapping panel dryness (TPD) (Fig. 2), resulting in the cessation of latex flow from the tapping cut. Towards the end of the economic life span

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of the tree, application of ethylene stimulant such as ethephon on the bark to get higher latex yield is a common practice in rubber plantations. Apart from the intensive latex harvesting, high dosage/frequency of ethephon application can also trigger the incidence of TPD up to 20% (Chrestin, 1989; Wu and Hao, 1993; Jacob and Krishnakumar, 2006). Fray and Jacob (1989) reported various morphological, structural, physiological and biochemical changes in association with the development of TPD.

Stimulating trees with partial TPD may yield latex for a shorter period but the flow of latex stops for ever (RRII, 2007). However, the possible mechanisms that trigger partial or complete cessation of latex flow in the TPD affected trees are still obscure. Paomod *et al.* (2008 and 2010) suggested that the deposition of p-protein and definitive callose in the sieve tubes restrict the downward transolocation of metabolites.

Protein storing cells (PSC) are elongated or rectangular parenchymatous cells found in phanerogams (Yuey *et al.,* 2007). These cells are rich in protein but devoid of starch, lipids and tannin deposition. The PSC are also known as albuminous cells, strasburger cells or myelin–like structures as per earlier reports (Alfieri and Evert, 1968; Sauter *et al*., 1976; Wu and Hao, 1986; Hao and Wu, 1994) and are in close contact with the sieve elements of the secondary phloem. Functionally these cells are similar to that of companion cells with respect to the bi-directional movement of nourishments (Ghouse and Mohd Yunus, 1975). Studies on the occurrence, its functional significance and distribution pattern of PSC in *Hevea brasiliensis* in response to TPD and ethephon stimulation are discussed in this paper.

## MATERIALS AND METHODS

Twenty-year-old trees of the *Hevea* clone RRII 105 from the Rubber Research Institute of India, Kottayam, Kerala were selected for the study. Bark samples were collected at 150 cm height from six trees each from healthy trees under tapping, TPD affected trees on rest and TPD affected tapping trees applied with 5% ethephon (bark application on monthly basis). In the case of stimulated trees, bark samples were collected six months after stimulation. The samples were fixed in formalin-acetic acid-ethyl alcohol (FAA) (Johansen, 1940) and sections at different planes *viz*., transverse, radial longitudinal and tangential longitudinal planes of 40-50 μm thickness were cut with a sliding microtome. The sections were stained for general histology with Toluidine blue O (O'Brien *et al*., 1964); acid fuschin (McCully, 1966), Coomassie brilliant blue (Mazia *et al*., 1953 ) and Amido black 10B (Weine, 1957) for protein localization; Mercuric chloride bromophenol blue (Mazia *et al*., 1953) for p-protein, lacmoid (Cheadle *et al*., 1953) for callose, Congo red for definitive callose and cytoplasm (Samuel, 1966) and phloroglucinol –HCl for lignin (Jensen, 1962). Observations were taken under a Leica Diaplan compound microscope attached with Leica Q win V3 image analysis system.

The soft bark tissues were homogenized with 0.1 M sodium phosphate buffer (pH 6.5) for extracting the soluble proteins. The supernatant collected from the homogenate was treated with cold acetone (1:1) overnight at $4^0$C for precipitating protein and removing other impurities from the crude extract. The precipitated proteins were re-dissolved in 0.15M $NaCl_2$ and the protein

**Fig. 1**

**Fig. 2**

Fig. 1. Healthy tree under tapping
Fig. 2. TPD affected tree

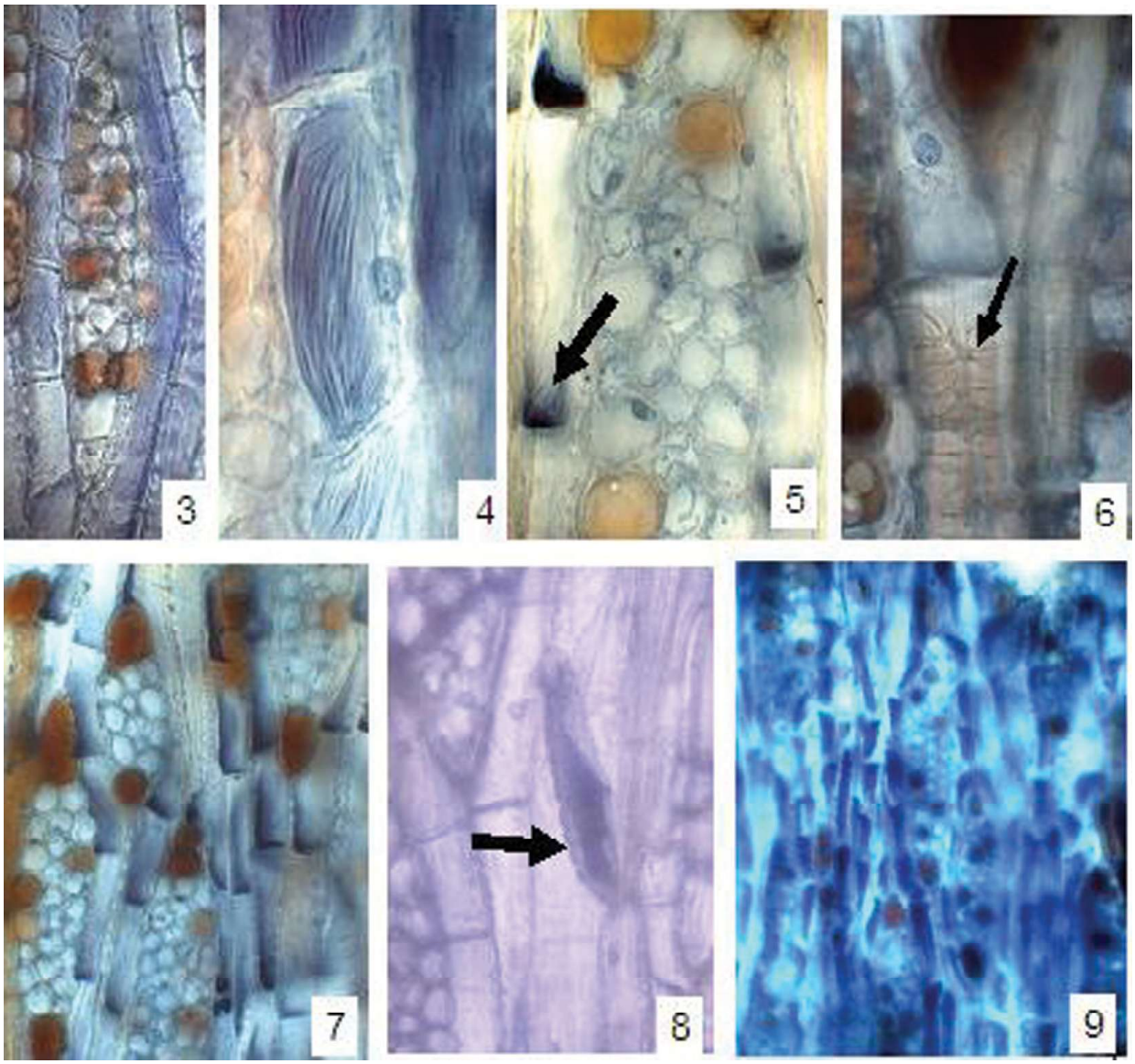
content was estimated following the method of Bradford (1976).

## RESULTS

The protein storing cells (PSC) are distributed in the parenchyma cells, sieve elements and phloic rays in the bark tissue of the rubber trees under tapping. In the inner soft bark region these cells have dense cytoplasm with fibrillar proteinaceous materials parallel to the long axis (Figs. 3, 4). These cells are uninucleate with prominent nucleolus placed in the centre or in the lateral position. PSC are uni or bilayered with frequent plasmodesmatal connections between the adjacent cells *viz.*, sieve tubes, phloic rays, parenchyma cells or other protein cells. The PSC are rectangular or square in shape and the size is similar to that of normal parenchyma cells. The laticifers were oriented in definite rows within the bark tissue in the form of articulated and anastomosing net work, with definite intercellular connections between the phloic rays, where as such inter connections were not observed between protein storing cells.

In the hard bark region, most of the tissue including the laticiferous system were less active or disorganized and sclereified. The fibrillar contents in the PSC became less dense, compartmentalized or dispersed with few deeply stained granular materials. In the later stage the proteinaceous material within the PSC was marginalized to one of the lateral walls leaving large unstained area within the cell (Fig. 5). At this stage the fibrillar content was more pronounced and the nucleus was shifted adjacent to the cell wall and some times even away from the protein condensed area surrounded by rind of peripheral cytoplasm. As the cell senescence proceeds, the protein became feebly stained or completely disappeared followed by the initiation of lignification process (Fig. 6). The nuclei with prominent nucleolus were present in the cells even though the cells were not stained for protein. It was also observed that PSC were devoid of tannin, starch or calcium oxalate crystals, whereas the normal parenchyma cells undergoing senescence showed tannin or crystals as occlusion.

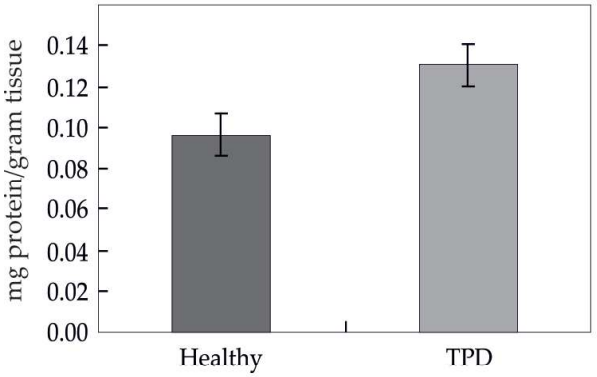
Deeply stained PSCs were observed in the TPD affected bark with comparatively thin soft bark similar to that of healthy trees. The cells were arranged in more than three rows around the phloic rays or in association with cambial zone cells (Fig. 7). Normal parenchyma cells adjacent to the PSC possess tannin content and crystals. The ray cells close to each other were separated by a layer of parenchyma cells with proteinaceous materials. Frequent plasmodesmatal connections were observed between the lateral walls of the protein cells as well as the terminal cells of the rays. Sieve plate was observed adjacent to protein storing cells distinctly but clear




Figs. 3, 4. Healthy bark fibrillar arrangement of protenaceous material in the protein storing cells: X 240; X 630
Fig. 5. The fibrillar content of PSC become less dense (at arrow) in the hard bark region X 240
Fig. 6. Lignification in PSC X 630
Fig. 7. PSC in the TPD affected bark X 240
Fig. 8. P-protein (at arrow) in sieve tube X 630
Fig. 9. PSC arranged in many layers in the TPD bark after Stimulation X 150

demarcations of the sieve tubes were not evident. P-protein was localized nearer to the sieve plates of the sieve tubes (Fig. 8).

PSCs were multi-layered in the stimulated TPD affected area (Fig. 9). Certain portions of the soft bark were devoid of sieve tubes and rays but mostly occupied with PSC. The plasmodesmatal connections between these cells were also more prominent. PSC have frequent




plasmodesmatal connections with the terminal cells of multiseriate rays where as these connections were not found across uniseriate rays or those cells filled with tannin.

The analysis of protein indicated that the bark tissue of trees with TPD symptoms had comparatively higher amounts of protein than that of healthy trees (Fig.10). The protein content was significantly increased in the tapping panel region of partial TPD affected trees. However during the initial stages of TPD, appearance of more proteins in the bark tissue was evident as a consequence of the abiotic stress response.

Formation of more parenchymatous tissue with varying shape and orientation were observed in the TPD bark under stimulation. These parenchyma cells act as protein storing cells and devoid of intercellular connections with phloic rays. The intercellular connection between these cells were more prominent than the connections between the PSC and phloic rays.

## DISCUSSION

It has been reported that the downward translocation of photosynthates through the sieve elements during the initial period was restricted due to the deposition of p-protein where as during the later stage was noticed the deposition of definitive callose in the TPD affected bark tissue of *H. brasiliensis* (Pramod *et al*., 2008; 2011). As a result the tissue present in the TPD affected zone including cambium was under starvation for adequate metabolites for the synthesis of latex (Gomez, 1982; Pramod, 2007). When the bark tissues were subjected to stimulation, the wet (healthy) areas in the TPD affected bark under starvation were forced to produce latex for a short period (RRII, 2007). During this time the plant may take alternative pathway to get the required metabolite precursors for the biosynthesis of latex, particularly through radial transport (Sauter and Witt, 1997). This phenomenon may trigger the abnormal functioning of the cambium leading to the formation of more number of parenchymatous tissue (Yamamoto and Kozlowski, 1987; Pramod, 2007), and the formation of abnormal tissue in the bark associated with alterations in tissue orientation. Occurrence of more PSCs in clusters was observed in the stimulated tapping panel that also had TPD. The formation of PSCs in abundance may be assumed as an independent function in the transport of metabolites under such conditions.

Sauter *et al*., 1976 reported that the increase in nuclear area with prominent nucleolus, dense cytoplasm and fibrillar orientation of protenaceous material, abundance of mitochondria of albuminous cells *etc*. were indications of increased physiological activity leading to translocation events in gymnosperms. The transcellular strands developed by p-protein in the sieve tube sometimes transformed into longitudinally oriented strands in response

to injury facilitating long distance transport due to the peristaltic waves produced by their rhythmic contraction (Evert *et al.*,1969). Both p-protein and PSCs have more or less similar fibrillar orientation and density when the bark tissues are under stress. As the functioning of sieve tubes in the TPD affected area are regulated by thick deposition of definitive callose in the thin strip of inner bark, PSC with large plasmodesmatal connections between cells may function as an auxiliary pathway for downward translocation of metabolites. Absence of starch, lipid, tannin or calcium oxalate crystals in PSC also supports the function of transportation. Radial transport of the metabolites to the TPD affected area was enhanced by the increase in the density of rays together with cell enlargement (Pramod, 2007). In general, plants may switch on to alternative strategies for the survival in changing environments in order to over come the prevailing stress situations as reported by Edreva *et al*., 2008.

It has already been reported that the bark tissue of healthy trees of *Hevea* possesses p-protein in the sieve tube (Wu and Hao, 1990; Pramod *et al.,* 2008) and fibrillar proteinaceous material 67 kDa in the parenchyma cells (Tian *et al.,* 1998). Moreover, these two types of proteins are structurally different or revealed by its specific stainability. Both these proteins are produced in abundance when there is stress so as to increase the mobilization of metabolites to the site of necessity under acute stress situations. Dian *et al.* (1995) also noticed excessive formation of proteins in association with TPD. The formation of fibrillar protein in the parenchyma cells also support translocating tissue in the bark to remain active.

In addition to the protenaceous materials in the PSC (67 kDa), plants possesses vegetative storage proteins (VSPs) ranging from 15 to 45 kDa suggested to be as storage reserves (Wu and Hao, 1987). Dian *et al*. (1995) reported major changes consisted of a dramatic increase of a 26 kDa and 45 kDa protein and minor changes affected in 55 kDa, 34 kDa and 21 kDa proteins in TPD affected trees. This implies that the 67 kDa protein reported to be present in the PSC of TPD trees of *Hevea* are different from VSP and may have some specific role when the plant is exposed to severe stress.

Zeigler (1964) reported the formation of albuminous cells in large quantity associated with unfixed rhythm in the cambial activity of gymnosperms. The cambial activity and the basic alignment of the tissue could be altered owing to TPD and stimulation in *Hevea* (Pramod, 2007). Hence, excessive formation of definitive callose (Pramod *et al*., 2008), p-protein (Pramod *et al.,* 2010) and protein storing cells in the TPD affected bark tissue may be a consequence of altered cambial activity including frequent transformative divisions.

The periclinal divisions of the fusiform initials of the cambium zone give rise to sieve cells, protein–storing cells and phloem parenchyma cells (Ghouse and Mohd.Yunus, 1975) whereas the ray cells are originated from ray initiates of the cambium (Crafts and Crisp 1971). Barnett (1974) believed that PSCs are part of rays that lies on the upper and lower margins of the central core constituted of ray parenchyma cells. Aronoff *et al*. (1974) reported that PSCs may be derived from both fusiform and ray initials in the cambium. The considerable increase in the production of

PSCs associated with less number of sieve elements in TPD affected and stimulated bark, in the present study, revealed that the origin of these cells is from fusiform initials as reported by Ghouse and Mohd.Yunus, 1975).

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