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Structural modification of phloic rays in *Hevea brasiliensis* with reference to tapping panel dryness and stimulation

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Abstract

Hevea brasiliensis, the prime source of natural rubber, when tapped intensively showed the symptoms of gradual cessation of latex flow from the tapping wound and this phenomenon is termed as tapping panel dryness (TPD), leading to a number of structural deformations in the bark tissues. The unproductive bark thus formed due to TPD is subjected to ethephon stimulation resume latex flow for a period. The study was initiated to trace the structural modifications occurred in phloic rays as an alternative route for translocation under necessity. The dimension of phloic rays also showed significant variation in TPD trees in comparison with both healthy stimulated trees. A decrease in length and an increase in width of phloic rays were evident in TPD affected trees over healthy trees. Average height of ray (µm) in the bark of healthy, TPD affected, unaffected zone above the TPD affected area and TPD panel under ethephon was 495, 259, 416 and 285 respectively. In healthy trees, 57 per cent of the rays fall in the stratified height class of 300-500 µm but in TPD trees, 78 per cent of the phloic rays is having a height less than 300 µm. The average width of the ray measured 56.81 and 74.25 µm respectively for healthy and TPD trees. In healthy trees 61 per cent of the ray falls under width strata of 40-60 µm and in TPD trees 68 per cent is in the 60-80 and 24 per cent in 80-100 µm width strata. For the production of latex from unproductive bark of TPD tree on stimulation, adequate nourishments is being mobilized to the site of action by strengthening radial transport system in the affected area.

Keywords: Ethephon, *Hevea brasiliensis*, phloic rays, tapping panel dryness

Introduction

In *Hevea brasiliensis*, the prime source of natural rubber, latex is formed in laticiferous tissue present in the bark and its harvesting is done through controlled wounding of the bark called tapping (Fig. 1). When rubber trees are subjected to intensive tapping, particularly in high yielding clones, the cessation of latex flow from the tapping panel was observed and is designated as tapping panel dryness (TPD; Fig. 2). It has been reported that in mature plantations more than 20 per cent trees turned unproductive due to TPD leading to considerable reduction in the revenue (Jacob and Krishnakumar, 2006). Common morphological symptoms and structural abnormalities reported in association with TPD are bark abnormalities such as drying, browning, thickening, cracking, flaking *etc*., (Fay and Jacob, 1989). Even though different reasons were attributed to this century old problem, till now no remedy has been found out other than few management practices through grafting (Premakumari *et al*., 1996) or debarking of the unproductive bark from the affected panel of the tree (Thomas *et al*., 1998).

One of the common cultural practices for getting better latex yield particularly during the end of the economic life span of the tree is the application of ethephon on the tapping panel (Jetro and Simon, 2007). Low frequency tapping is being recommended with ethephon application even in the initial stage of tapping itself (Karunaichamy *et al.*, 2001). It has also been reported that excessive application of

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Fig. 1. A healthy rubber tree under tapping

Fig. 2. Rubber tree affected by tapping panel dryness with warty out growth

stimulant together with intensive tapping can induce TPD. Application of ethephon on the TPD affected bark may yield a little latex during the initial period and gradually stops with in a shorter duration (RRII, 2007). The downward translocation of photoassimilate through sieve elements in TPD trees were restricted due to the thick deposition of definitive callose and p-protein (Pramod *et al*., 2008, 2011a), and the plant mobilises available photo-assimilates alternatively through radial transport system for latex synthesis. The present article describes the structural modifications occurring in the phloic rays in TPD affected and ethephon applied TPD affected area of the bark.

Materials and Methods

The experiment was conducted on 20 year old trees of *Hevea brasiliensis* (clone RRII 105) planted in Rubber Research Institute of India at Kottayam, Kerala, India. Six trees each were selected to represent healthy trees, trees affected by TPD, TPD trees applied with ethephon (5% applied on the tapping panel at monthly interval for a period of six months). Samples of bark with intact cambium were collected from tree trunk with a sharp chisel and the tissues were fixed in formalin-acetic acid-ethyl alcohol (FAA; Johansen, 1940). Tangential longitudinal sections (TLS) at 20-40 µm thickness were taken using Leica SM2010R sledge microtome. Sections were stained with Toluidine blue O (O'Brien *et al.*, 1964) for general histology; lacmoid (Cheadle *et al*., 1953) for callose and tannic acid-ferric chloride for tannin (Johansen, 1940), Coomassie brilliant blue (Mazia *et al*., 1953) and Amido black 10B (Weine, 1957) for protein and prepared the slides for microscopy. Observations and measurements were taken using a Leitz Diaplan compound research microscope attached to Leica Qwin V3 image analysis system. The data was analysed statistically.

Results and discussion

The cambium in *Hevea brasiliensis* is non-storied with elongated fusiform and ray initials (Fig. 3). In tangential view of the cambium, ray initials usually appear as isodiametric in outline and grouped into a definite fusiform shaped pattern interspersed among vertical rows of fusiform initials. Multiplicative divisions (transverse and vertical anticlinal) increase the number of initials leading to the growth of rays both in height and width, but more contributes to ray height than in width. Functionally productive tissue in the inner bark is termed as soft bark and the outer bark is termed as hard bark which is less active or unproductive in nature.

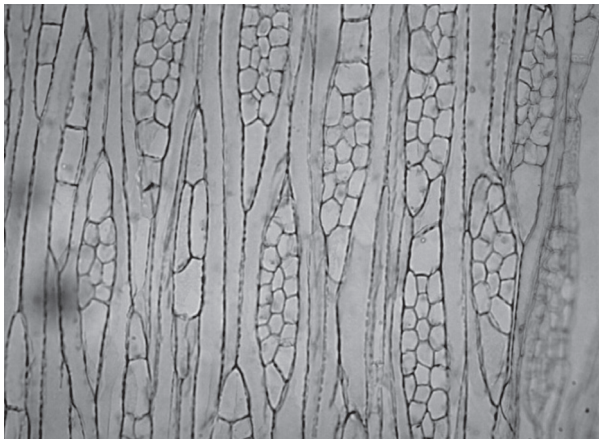

Fig. 3. Non-storied orientation of fusiform and ray initials in the cambium of healthy tree

Phloic rays, the route for radial transport of nutrients, in the soft bark of healthy trees of *Hevea brasiliensis* have a non-storied alignment of multiseriate rays with 1-4 uniseriate isodiametric terminal cells on either side (Fig. 4). They are oval, elongated or as dumb-bell shaped due to a sequence of unicellular array in between the multiseriate aggregations. The multiseriate region is composed of 4-5 layers of more or less angular cells without intercellular spaces. Phloic rays in the soft bark contiguous to cambium are devoid of phenolic contents or druse type crystals whereas in the hard bark region, particularly towards the exterior, the cells were more shrivelled and possessed little quantity of reserve metabolites, filled predominantly with phenolic contents or crystals.

The TPD affected trees and TPD affected area stimulated with ethephon showed significant difference in their ray height, width, intra ray cell diameter, inter ray distance and number of cells that constitute the width of the ray (Table 1). A reduction in height and an increase in width was observed for phloic rays in TPD trees over healthy trees. Height of the ray (µm) was 495, 259, 416 and 285 respectively for healthy trees, TPD affected trees, unaffected area above the TPD affected panel and TPD affected panel applied with ethephon. Ray height in healthy trees and unaffected area of TPD trees even extended above 1000 µm (Table 2) whereas highest value for TPD affected and stimulated trees was recorded up to 500 µm. In the case of healthy trees, more than 55 per cent of the rays were having a length of 300-500 µm. Owing to TPD, 78 per cent of the rays showed the height below 300 µm (Table 2). The ray width was increased due to TPD and the values ranged from 56.81 µm (healthy tree) to 74.25 µm in TPD affected area. In the unaffected area of the TPD tree, average width of the ray was 66.43 µm indicating that it is intermediate to healthy trees and TPD affected panel (Table 1). For healthy trees 60.9 per cent of the rays fall in the width class 40-60, while in TPD panel and unaffected zone of the TPD tree, 68 per cent are in the 60-80 µm width class. As a result of ethephon application, ray width was increased; 54.4 per cent in 60-80 and 33.3 per cent in 80-100 µm was noted in ethephon applied trees. Intracellular diameter had increased from 20 µm in the healthy tree to 27 µm in the TPD bark. In healthy trees 41 per cent comes under 15-20 µm strata and higher values recorded for TPD (40%) and stimulated (36%) trees in the range 20-25 µm. The inter-ray distance is 61 and 62 µm for healthy and TPD bark while the value recorded for TPD bark applied with ethephon is 46 µm. Number of cells constituting the width of

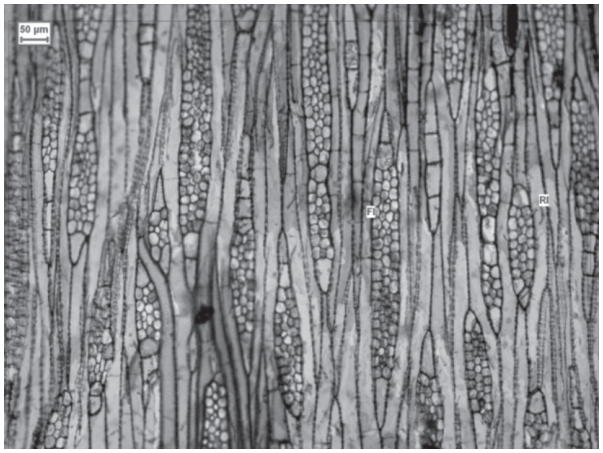

Fig. 4. Phloic rays in the soft bark of healthy tree

Table 1. Ray characteristics in healthy, TPD affected and ethephon applied trees of *Hevea*

Ray characters (µm)	Healthy	TPD	Unaffected area of TPD tree	Ethephon on TPD bark
Height	495.88[a]	258.71[c]	416.37[b]	285.27[c]
Width	56.81[b]	73.78[a]	66.43[a]	74. 25[a]
Intra ray cell diameter	20.47	24.12	26.78	22.88
Inter ray distance	61.78	62.98	55.44	45.52
No of cells constitute width	3.13[b]	3.93[a]	3.33[b]	3.71[a]

Table 2. Stratified data on ray parameters (percentage) from different treatments

Parameters & strata (µm)	Healthy tree	TPD tree	Unaffected area of TPD tree	TPD bark applied with ethephon
Height				
<300	7.0	77.7	27.4	59.3
300-400	26.8	20.4	28.3	35.6
400-500	29.6	1.9	24.5	5.1
500-600	15.2	-	10.4	
600-700	10.7	-	1.9	
700-800	3.7	-	2.8	
800-900	2.5	-	-	
900-1000	1.1	-	1.9	
>1000	3.4	-	2.8	
Width				
<40	7.0	-	2.8	-
40-60	60.9	7.9	20.2	12.3
60-80	26.2	68.3	68.8	54.4
80-100	5.5	23.8	8.3	33.3
>100	0.4	-	-	
Inter ray distance				
<25	10.0	7.9	14.4	20.4
25-50	38.6	27.0	39.7	43.4
50-75	27.1	42.9	24.0	24.3
75-100	10.0	14.3	13.0	6.6
100-125	10.0	4.8	3.4	4.6
125-150	1.4	-	5.5	0.7
>150	2.9	3.2	-	-
Intracell diameter				
<15	6.6	-	2.5	7.0
15-20	40.7	19.3	24.2	28.4
20-25	38.4	40.4	23.3	35.8
25-30	12.4	27.5	27.5	18.3
30-35	1.6	9.2	7.5	5.7
>35	0.4	3.7	15.0	4.8

phloic ray was higher in the TPD bark (3.93) than that of healthy tree (3.13).

A reduction in length and an increase in width of phloic rays (Fig. 5) were observed in the recently differentiated mass adjacent to the cambium of TPD affected trees. The density of rays in the TPD affected bark was also increased as a result of transformative divisions in the fusiform initials. The small initials thus formed divide transversely to form uniseriate rays with larger cells in the affected bark. Sometimes these cells act as a link to connect adjacent rays vertically. The newly formed ray cells are 3-4 fold larger than the remaining ray cells with prominent nucleus and deeply stained nucleolus, and appear as rounded to elongate or as irregular in outline with intercellular spaces (Fig. 6).

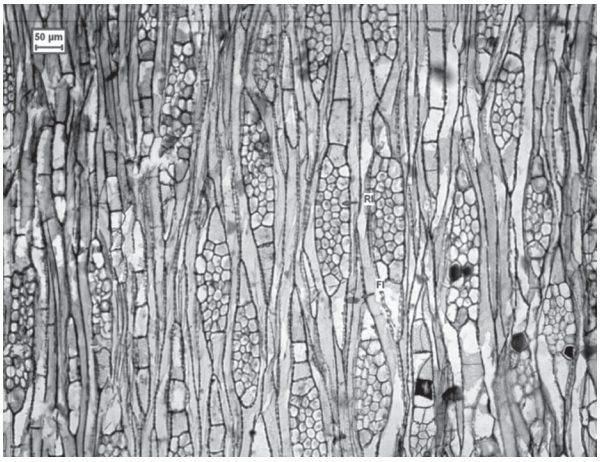

Fig. 5. Shortening and widening of rays in TPD trees

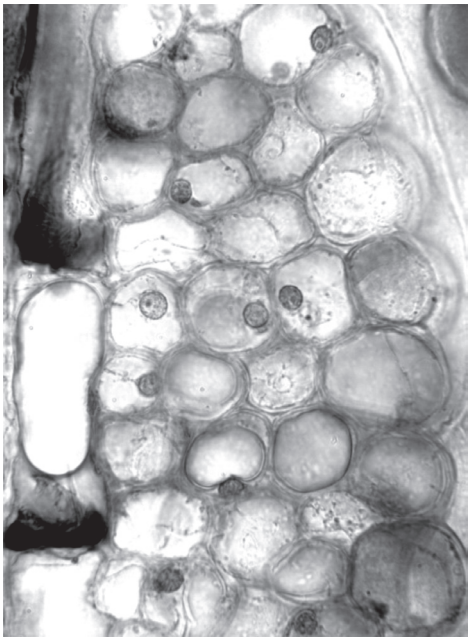

Fig. 6. Phloic ray in the TPD trees with dense cytoplasm and prominent nuclei

New ray initials also arise through few or frequent septation of an entire or a part of fusiform initial resulting in the formation of a layer of narrow elongated or bead like oval to oblong cells as a part of phloic ray. These cells have thick outer wall and maintain frequent connections with the adjacent parenchyma cells and may possess druse type crystals or phenolic contents. In some cases a small portion of the fusiform derivatives known as sheath cells often appears along the ray margins in the soft bark of TPD affected trees. Intrusive growth of cells into the phloic rays was quite frequent in the multiseriate rays (Fig. 7).

Fig. 7. Intrusive growth of parenchyma into a ray

Abnormal structure of phloic rays was more pronounced in the TPD affected tree with warty out growth in the affected area (Fig. 8). In the TPD trees with warty out growth, the outline of the ray varied from circular, oval, elongated, horse shoe shaped, triangular or aggregate which were en-sheathed in many instances by elongated or isodiametric parenchymatous cell (Figs. 9 & 10). Uniseriate rays constituted of uneven cells existed either independently or in close association with the multiseriate rays.

Application of ethephon on TPD affected panel makes it productive for a short period. After 2-3 months, a complete cessation of latex flow from the tapping panel was observed. Deformation for phloic rays were more pronounced in such trees than unstimulated TPD trees (Fig. 11). This occurs due to the enlargement of procumbent cells of the ray system, increases the intercellular spaces or by the adherence of modified cells derived from alternative sources to the apical or lateral region of the ray (Figs.12 &13). The phloic ray thus formed had an outline without uniformity in cell size or shape. The stout upright cells had deeply stained cell walls with frequent intercellular connections and the remaining cells of the ray system had thick cell wall, abundant cytoplasm with prominent nuclei and nucleolus (Fig. 13). Axial parenchyma cells in many loci retained meristematic activity and many of the cells,

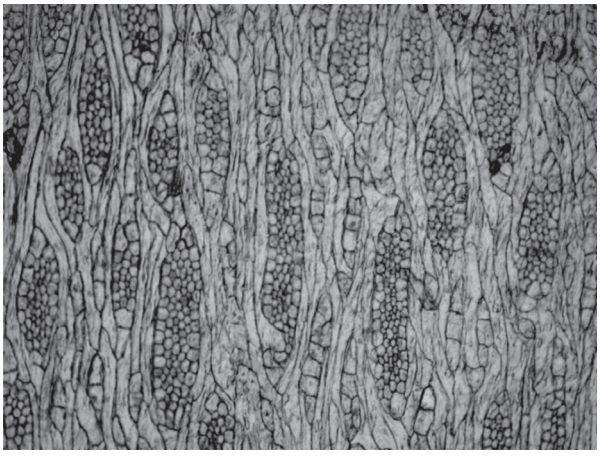

Fig. 11. Shortening and widening of phloic ray in the stimulated bark

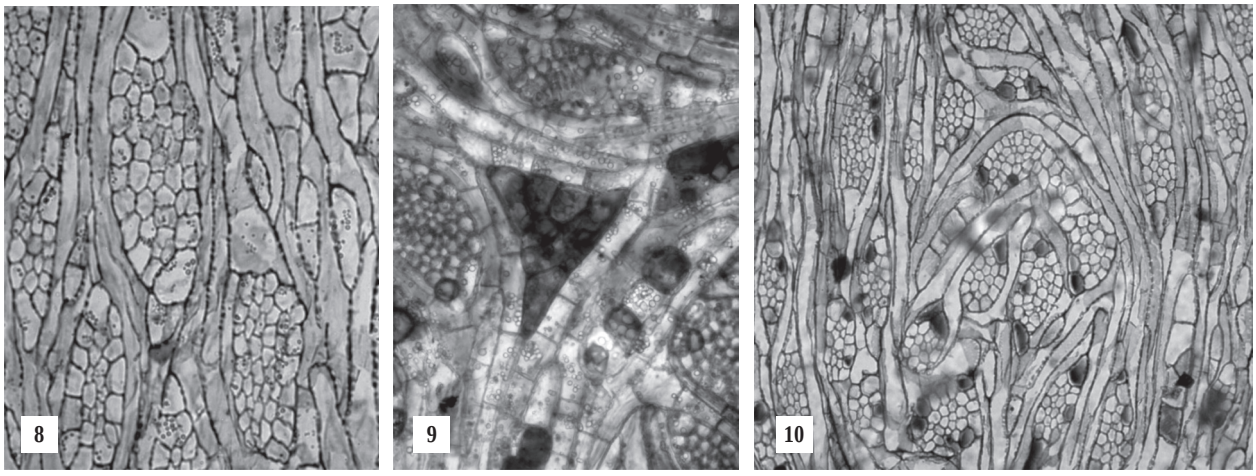

Fig. 8, 9, 10. Ray characteristics of TPD affected tree with warty out growth

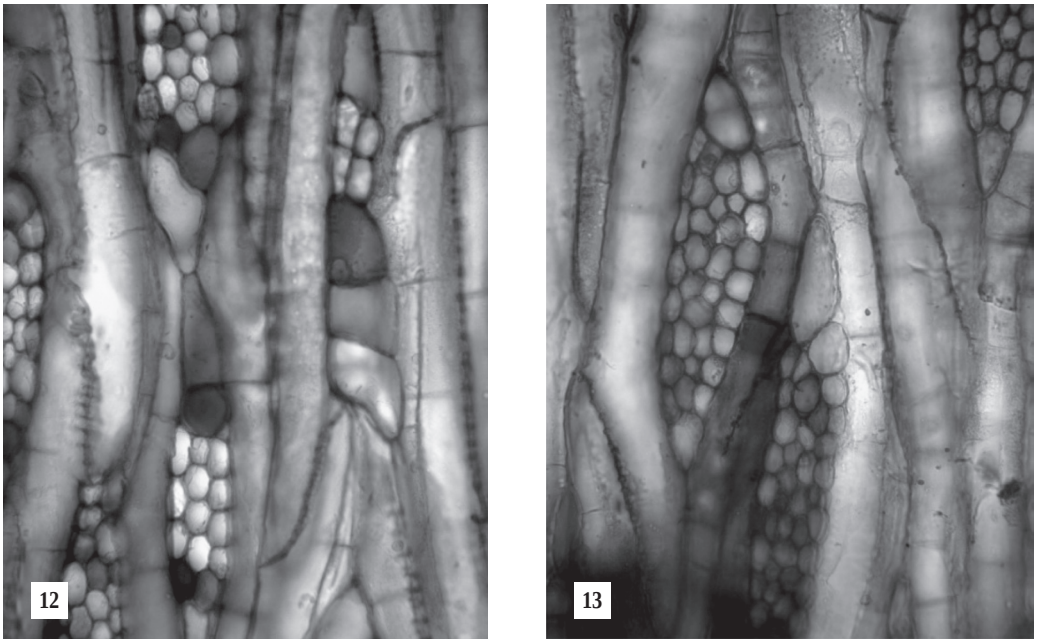

Fig. 12, 13. Parenchymatous tissue with varying shape intruded into or adhered to the phloic ray sytem

particularly the terminal cell possessed phenolic contents.

TPD affected area applied with ethephon triggered deformations in size, shape and orientation of phloic rays with respect to different loci within the affected panel. A definite terminal cell may or may not be present on either side of individual rays but a group of rounded to oblong cells with comparatively larger size than the ray cells aggregate and are found adhered to the terminal or lateral part of the phloic ray (Fig. 14). The aggregated cells thus adhered showed frequent divisions in anticlinal, periclinal or oblique manner to form a file arrangement for the daughter cells produced (Fig. 15). In some of the cases individual cells of the ray were characterised by thick wall, dense

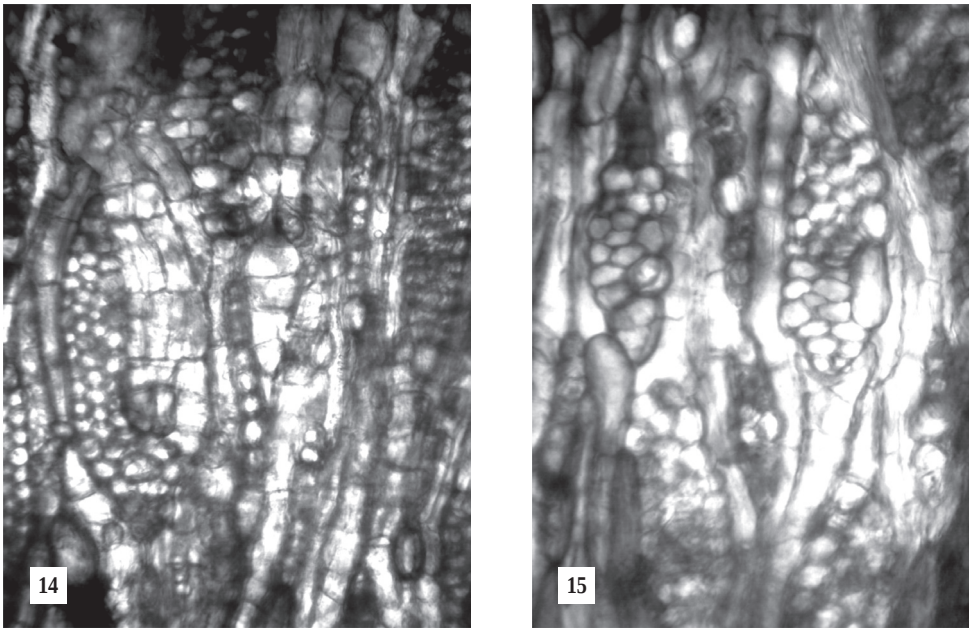

Fig. 14, 15. Phloic ray had lost its size and shape following to TPD and stimulation

cytoplasm and prominent nucleus with nucleolus, arranged with or without intercellular spaces. The cytoplasm as a whole stained feebly but the margins were deeply stained in some of the ray cells. Phenolic contents and druse type crystals were found deposited in some of the ray cells. Intrusion and division of sieve tubes or parenchyma cells either increase the area of phloic ray or split partially or completely into two parts. A layer of narrow but laterally elongated parenchyma cells with prominent nucleus were adhered laterally to the ray system to make it wider. At different loci in the panel, the ray cells were difficult to demarcate from the surrounding cells as there are many with intermediate shapes. Three to four fold larger cells than the axial parenchyma with rounded shape were arranged in a row and the terminal cell maintains connection with adjacent parenchyma cells possessing fibrillar protein (Protein storing cells; Fig.16). In certain loci of the affected panel, protein storing cells are in abundance where the ray cells are not frequent.

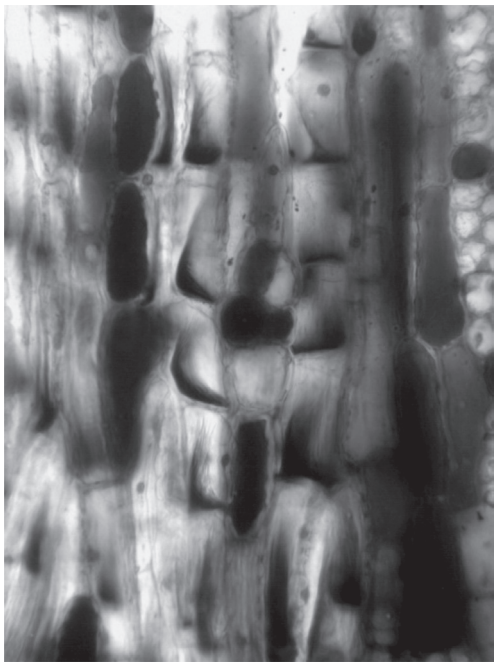

Fig. 16. Parenchymatous tissue possessing fibrillar protein

In woody plants, when the resources are allocated to cambium in adequate quantity seems to be an indication of good health of the plant (Savidge, 1985, 1996; Steeves and Savidge, 2000). The activity of cambium and subsequent tissue differentiation require high levels of energy and the requisite for the same is made available mainly from the stored reserve metabolites in the subsequent cells (Evert *et al*., 1972; Berlyn and Battey, 1985). Downward translocation of photo-assimilates through the sieve tubes to the TPD affected bark in *Hevea brasiliensis* is restricted to a certain extent due to the deposition of p-protein and definitive callose on the sieve plate (Pramod *et al*., 2008, 2011a) leading to certain abnormality in the division and function of the cambial initials (Pramod *et al*.. 2011b). Under this situation, the cambial derivatives give rise to more parenchymatous tissue and phloic rays through transformative division of the fusiform initials. In *Populus tremuloides*, the cambium started to produce more parenchymatous tissue when the bark was severed either during the dormant or growing season (Evert *et al.*, 1972). The above reports revealed that the division of the cambial initials is sensitive to stress conditions developed through various factors such as nutrient scarcity, wounding, application of stimulant *etc*. and in many instances the alteration in the activity of cambium and subsequent tissue differentiation is irreversible too (Thomas *et al*., 2006; Pramod *et al*., 2011b).

The activity of cambium is an energy rich process and the major factors influencing cambial function includes stress due to mechanical, physical or physiological factors, and source-sink effects on carbohydrate translocation, allocation and utilization *etc*. (Berlyn and Battey, 1985). The tree has its own mechanism to thrive over the unfavourable situation by strengthening other routes of translocation to compensate the acute shortage of assimilates. The sieve tubes that differentiated from the fusiform initials in TPD affected trees of *Hevea* does not have much role to play under the stress condition (Pramod *et al*., 2008, 2011b) as these initials are eventually able to divide unequally and can even give rise to new ray initials for alternate radial routes for food supply (Sauter, 2000).

Initiation of new rays, regulation of ray size and determination of ray shape are governed by a number of factors, among which ethylene has a major role that can change the activity of cambium and differentiation of various tissue from the derivatives cut off from the cambium (Baker, 1979; Yamamoto and Kozlowski, 1987; Iqbal, 1994). In

the TPD affected and stimulated loci of *Hevea*, more number of phloic rays were produced through transformation of fusiform initials in the cambium as a consequence of high amount of ethylene accumulation followed by intensive tapping or the external application of ethylene on the bark (Krishnakumar *et al.,* 2008; Thomas *et al*., 2012). Lowerts *et al.* (1986); Mattoo and Aharoni (1988); Savidge (1988) and Lev-Yadun (1995) reported that ethylene is involved in transformation of fusiform initials to ray initials and the un-proportionate formation of enlarged unicellular rays and formation of aggregate rays are indications of abnormal differentiation of this tissue in the affected patch of *Hevea* bark. The increase in the ray diameter did not increase the ray height, revealing that the dimensional changes in the rays occur not with cell enlargement, but by division, addition of ray initials and ray fusion (Philipson *et al.*, 1971). The increase in ray dimension in the TPD bark applied with ethephon was mainly due to the adherence of modified parenchyma cells to the ray system which might strengthen the transportation route.

Latex vessels in the TPD affected bark are found to be non-functional as no latex was oozing out from the bark on tapping. It is evident from the earlier experiment that these latex vessels were not actually dried but remain passive under severe starved situation and also capable to synthesise latex, provided there is a supply of metabolites in required quantity either through downward or radial transportation mechanism. In a healthy tree, the rubber biosyntheisis capability of laticifers is concentrated where phloic rays and sieve tubes actively transport metabolites (Sando *et al*., 2009). A major part of the nourishments required for the biosynthesis of latex within the latex vessels of TPD trees following stimulation are discharged through phloic rays. Subsequently, the development of more protein storing cells were observed in the TPD affected and stimulated bark (Thomas *et al.*, 2012) which also favour cellular mobilisation. Latex biosynthesis is possible even when the cambium is functioning abnormally. The functioning of cambium in the TPD affected area had altered in a permanent manner and under which the latex vessels differentiation may remain active. This indicated that cambial activity is more sensitive and fundamental requisite as far as plant life is concerned, and latex biosynthesis can be prolonged or suspended for a longer period.

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