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STRESS RESPONSE OF SIEVE TUBES DURING INTENSIVE TAPPING IN *HEVEA BRASILIENSIS:* AN ANATOMICAL PERSPECTIVE

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Major quantum of natural rubber is being recovered as an industrial raw material from the latex obtained on tapping the bark of *Hevea brasiliensis*, a deciduous tropical tree with an economic life span of about thirty years. Genetic improvement for achieving better attributes including high yield is one of the prime areas of research in this crop. It has been established that high latex yielding clones are vulnerable to a syndrome termed Tapping Panel Dryness (TPD), when the trees are subjected to intensive tapping. Cessation of latex flow from the tapping panel is the initial symptom, followed by the occurrence of a number of morphological, structural, biochemical and physiological changes resulting in complete drying of the bark. Even though different reasons are attributed for this century old problem, till now there is no remedy other than a few management practices. Sieve tubes, the prime channel for photoassimilate translocation in the bark of the tree that cater nourishments for latex biosynthesis exhibit an array of deformations following TPD. In this context, stress responsive plant deformations *viz.*, permanently altered cambial activity, intense deposition of definitive callose and P-protein, chaotic dynamic system in the sieve tube, peroxidase activity, protein storing cells and phytoplasma with respect to TPD are reviewed.

Keywords: Definitive callose, Dynamic system, *Hevea brasiliensis*, P-protein, Phytoplasma, Sieve tubes, Tapping panel dryness

INTRODUCTION

The tropical deciduous tree *Hevea brasiliensis*, yields natural rubber as latex which is collected from the bark of the trunk by a systematic wounding (Fig. 1) termed tapping. In each tapping, a thin shaving of the bark is removed by which interspersed laticifers are opened for initiating the flow of latex, leaving the cambium undisturbed. The cambial zone together with the inner soft bark left uncut in each tapping is called residual bark which is instrumental in healing and subsequent bark regeneration. After a period of time, uniform bark regeneration occurs if the cambium is not injured and repeated tapping in the same region is possible (Thomas *et al*., 1995). The quality and frequency of tapping influence the exploitation period of tapping panels, making the trees productive for a longer period.

In India, nearly one million hectares of land is under rubber cultivation of which more than 80 per cent is planted with the

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Fig. 1. Healthy tree of rubber under tapping

Fig. 2. TPD-affected tree showing warty outgrowths on the tapping panel

indigenously developed high yielding clone RRII 105 having better secondary attributes (Mydin, 2014). The RRII 400 series clones with better or comparable yield and secondary attributes are now gaining popularity and wide acceptance among growers (Mydin *et al*., 2011; Thomas *et al*., 2014). It has been established that high yielding clones, in general, are vulnerable to a syndrome termed 'Tapping Panel Dryness' (TPD) especially when the trees are subjected to tapping under high frequency (Sivakumaran *et al*., 1988; Premakumari *et al*., 1996; Thomas *et al*., 1998). The latex exuded in each tapping is to be synthesized in the drainage area of the tapping panel before the next tapping. In high yielding clones, the time taken to synthesize fresh latex is more than that in a low yielding clone and hence, a low frequency tapping system is recommended in the former for minimizing the incidence of TPD.

Prolonged dripping of latex followed by cessation of latex flow and drying of bark are the initial visible symptoms of TPD. The bark and wood of affected trees show many morphological, structural and biochemical changes such as drying of bark, bark flaking, development of warty outgrowth in the tapping panel (Fig. 2), discoloration of the freshly tapped bark, formation of occluded xylem, abundant sclereids in the bark, blocking of latex vessels with tyloses and intensive activities of respiratory and other enzymes such as acid phosphatase, ATPase *etc.* (Gomez, 1982; d' Auzac *et al*., 1989; de Fay and Jacob, 1989).

Economic life span of the rubber tree is about 30 years, and towards the end, application of ethephon on the bark as a stimulant is a common practice in rubber plantations (Jetro and Simon, 2007). It has been reported that about 30 per cent of the trees turn unproductive due to TPD over a period of time resulting in a substantial loss in revenue from the senile plantations (Jacob and Krishnakumar, 2006). Different reasons are attributed for this century old affliction among which much attention has been received for the physiological disorder as a result of intensive tapping, excessive application of yield stimulant or the association of viroid (Wu and Hao 1993, 1994; Ramachandran *et al*., 2000). The etiology still remains unknown and no remedy has been found out other than a few management techniques such as tapping rest for a period of time, grafting with healthy bark (Premakumari *et al*., 1996) and debarking of the unproductive bark (Thomas *et al*., 1998). Partially affected trees upon stimulation may yield latex for a short period but the flow stops forever later (Thomas *et al*., 2012a). The possible mechanisms that trigger partial or complete cessation of latex flow in the TPD trees are still obscure.

The anatomical, physiological and biochemical studies related to TPD reveal structural modifications such as substantial reduction in productive tissues within the bark, altered activity of cambium, alternative mechanism for strengthening the metabolite mobilization *etc*. (Pramod *et al*., 2008; 2011a, b; Thomas *et al.,* 2013). Among the bark tissues, the sieve tubes, the prime pathway for the downward translocation of photoassimilate is one of the major tracts which is considerably blocked (Thomas *et al*., 2012a). The present article summarizes the anatomical and histochemical changes pertaining to sieve tubes in the bark of *H. brasiliensis* following TPD and the effect of application of ethephon as a stimulant for yield enhancement.

Sieve tubes

Sieve tubes are one of the least understood cell types in plants. Sieve tube, one of the structural components of bark differentiated from the fusiform initials of vascular cambium is characterized by a long tube like structure with sieve plates on either end. The morphology of the sieve plate is important because the sieve plate has a major role on the regulation of flow in the sieve tube (Thompson, 2006). The sieve plate possesses sieve pores that is encircled by callose (β 1,3 glucan). Sieve tubes are highly vacuolated with a rind of cytoplasm near the cell wall, bounded with a tonoplast. The prime function attributed to sieve tube is the downward translocation of photoassimilate from its point of biosynthesis in the leaf to different parts of the plant.

Functionally active sieve tubes are turgid and photoassimilate translocation is under high hydrostatic pressure inside the sieve tubes with strongly thickened sieve element cell walls (Esau and Cheadle, 1958; Romberger *et al.*, 1993). To ensure symplast transport, phloem cell undergoes complex differentiation process during ontogenesis (Esau, 1969). Thomas *et al.* (2012b) reported the occurrence of a dynamic system in the sieve tubes of *H. brasiliensis* and its possibility of exerting pressure on the walls of sieve tubes. As the photoassimilate translocation is highly restricted in TPD-affected trees, the pressure developed in the sieve tube could be feeble and the force exerted by the surrounding tissues on the walls of sieve tubes is likely to be more, developing to unevenness for the sieve tube walls.

A comparison of sieve tube characteristics *viz.* length, diameter, density and grouping pattern of both healthy and TPD-affected trees (Fig. 3, 4) revealed that these characteristics of healthy trees are greater than the unaffected

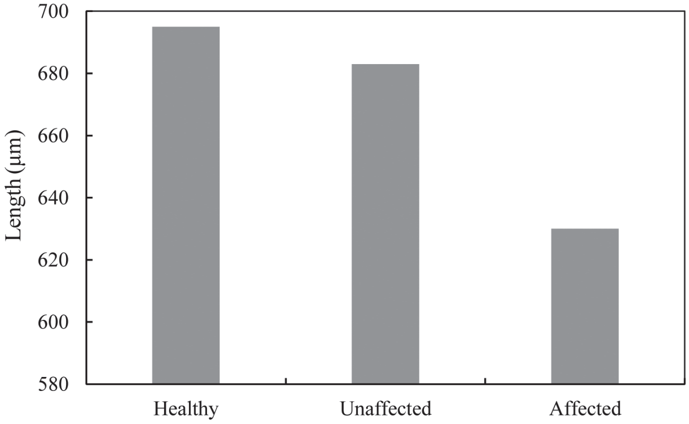


Fig. 3. Length of sieve tube in healthy and TPD-affected trees (unaffected and affected areas)

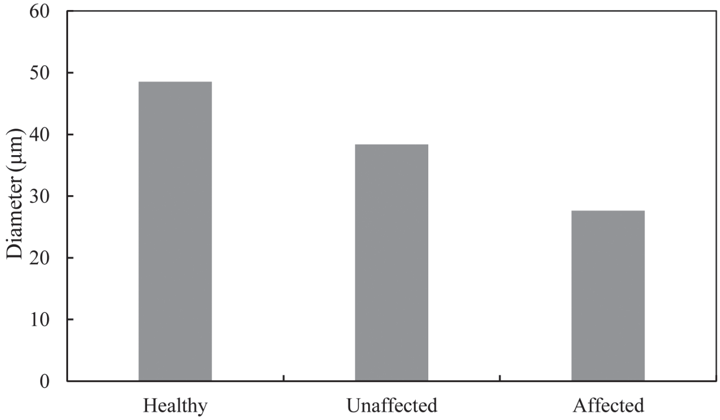


Fig. 4. Diameter of sieve tubes in healthy and TPD-affected trees (unaffected and affected areas)

area of TPD-affected tree, followed by the affected region of the tree (Gopal and Thomas, 2012).

The sieve element in the healthy tree was observed to be having a greater length in a range of 550-700 µm with an average of 695 µm. However, there was a considerable reduction in the length of sieve tube in the TPD-affected area with an average of 632 µm. The unaffected area of TPD tree showed an intermediate value of 683 µm. The average diameter of sieve tubes was 48.55, 38.37 and 27.63 µm respectively in healthy, unaffected region and affected regions of TPD trees. In healthy trees, sieve tubes are more or less uniform in outline throughout the soft bark whereas in the affected region of the TPD tree, the sieve tubes are disorganized and its shape showed a wriggling nature.

The highest density was recorded for healthy trees (55) followed by the unaffected area of the TPD tree (42) and the least for the affected area of the tree (33). In general, sieve tube characteristics showed significant clonal variation but within a clone, tree-to-tree variation was not evident (unpublished).

Latex biosynthesis occurs in the latex vessels which are tubular in nature and interconnected to form a net like appearance in the bark. They are distributed in definite concentric rings interspersed with other secondary phloem tissues. The nourishment required for biosynthesis of latex is routed primarily through the sieve tubes and phloic rays (Sando *et al.,* 2009), which can be attributed to the correlation between sieve tube characteristics and latex yield.

Latex yield is a complex phenomenon governed by a number of internal and external factors among which anatomical parameters mainly bark thickness and characteristics of latex vessels are significant (Pollinere and d' Auzac 1966; Premakumari, 1992). These parameters possessed positive correlation with latex yield of which latex vessels showed clonal variation also (Mydin *et al.,* 2011).

Sieve tube characteristics are also correlated with latex yield. A negative correlation between sieve tubes and latex yield was reported by Narayanan *et al.* (1973) whereas a positive correlation was observed by a number of researchers (Gunnery, 1935; Fernando and Tambiah, 1970; Assini *et al.*, 1998; Omman and Reghu, 2013). Sieve tube characteristics such as length, diameter and density (0.2 mm^{2}) were found to be positively correlated with latex yield (Table 1). The high yielding clone, RRII 105 recorded the highest sieve tube length (693.72 μm) and lowest for RRII 33 (408.72 μm) which is a low yielding clone. Average diameter of the sieve tubes of different clones ranged from 39.1 μm (RRII 33) to 48.2 μm (RRII 105). Maximum density for sieve tubes was recorded for clone RRII 105 (55) followed by PB 260 (50), RRIM 600 (44), RRII 33 (36), RRII 38 (34), RRII 118 (33) and RRII 208 (30).

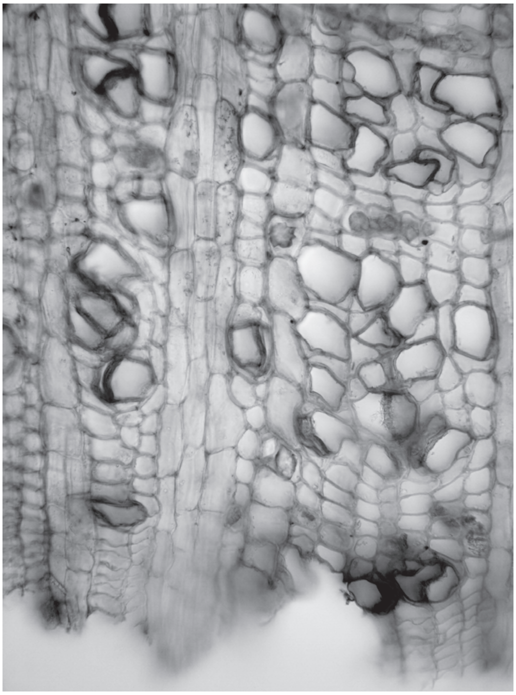
In many of the earlier studies related to evaluation of experimental clones of *H. brasiliensis*, sieve tubes were not taken into account as a structural trait related to latex yield because of the lack of a specific stain for the identification of sieve tubes, particularly in cross sectional view of the bark which provides information on density, diameter, grouping pattern *etc*. of sieve tubes. Both sieve tubes and latex vessels are arranged alternatively in the bark parallel to each other in the long axis of the tree trunk, and hence, its identification using general

Table 1. **Diameter, density and length of sieve tubes and mean yield of *Hevea* clones**

Clone	Average Length# (μm)	Average Diameter (μm)	Average Density	CV (%)	Mean yield* (g t^{-1} t^{-1}) over 5 years
RRII 105	693.7^{a}	48.2^{a}	55^{a}	28.3	56.8
RRII 208	596.8^{c}	42.1^{b}	30^{c}	23.4	51.2
RRII 118	510.1^{d}	41.11^{b}	33^{bc}	28.5	50.0
PB 260	621.5^{bc}	42.5^{b}	50^{a}	27.8	42.5
RRIM 600	594.0^{c}	42.3^{b}	44^{ab}	23.7	42.4
RRII 33	408.7^{e}	39.1^{c}	36^{bc}	24.1	15.8
RRII 38	633.0^{b}	40.2^{c}	34^{bc}	23.3	11.7

#Means followed by a common letter are not significantly different at $p<0.05$

*Saraswathyamma *et al.* (2000)



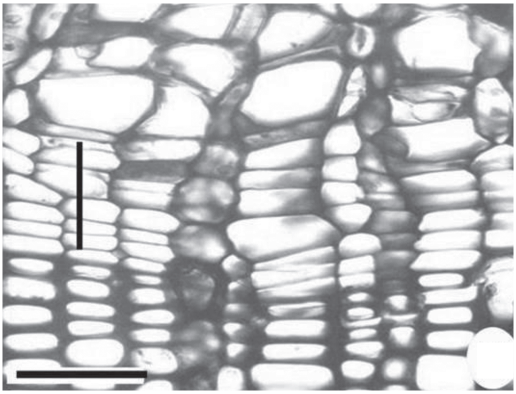
(Source: Gopal and Thomas, 2013)

Fig. 5. Sieve tubes stained with O-dianisidine

staining technique is difficult. Gopal and Thomas (2013) developed a method for staining sieve tubes in cross sectional view of *Hevea* bark using O-dianisidine (Fig. 5), a stain generally used for the localization of peroxidase enzyme. O-dianisidine stains the cell wall and sieve plate of the sieve tube reddish brown round the year. The cytoplasm of phloic rays in the bark, the tissue responsible for radial translocation, stains peroxidase with respect to season only (Gopal and Thomas, 2012).

Vascular cambium

The sieve tubes get differentiated from the fusiform initials of cambium. The cambium is non-storied in *Hevea*, characterized with elongated fusiform initials and has actively growing tips enclosing uni or multiseriate rays (Fig. 6, 7). The cambium exhibits seasonal activity and the structural characteristics associated with bark and wood are the reflections of this (Thomas *et al.*, 2002; Pramod *et al.*, 2011a). As a consequence of TPD, the rhythmic activity of cambium in *Hevea* is altered in a permanent manner that results in an increase in the density of cambial rays (Fig. 8) transforming fusiform cambial cells into ray cambial cells in the affected area of the tree. As a result, a considerable reduction in the



(Source: Pramod *et al.*, 2011a)

Fig. 6. Cross sectional view of cambium in its active phase

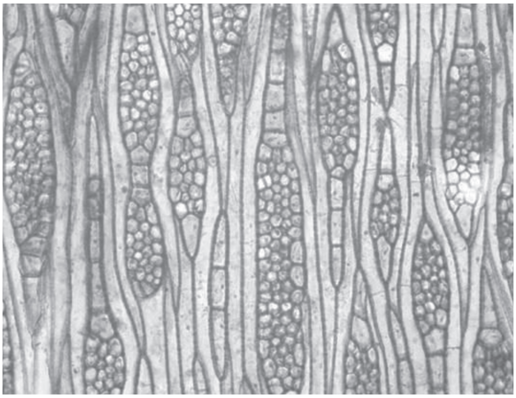
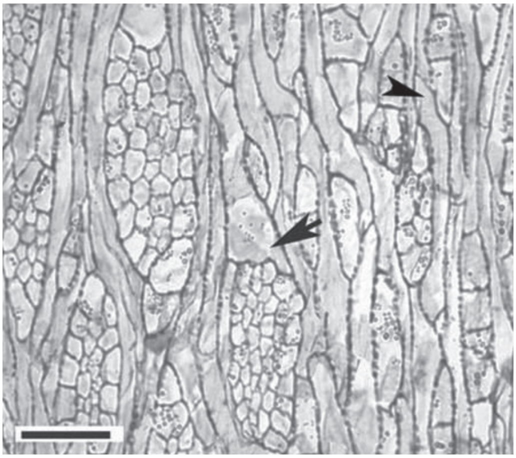


Fig. 7. Tangential longitudinal view of cambium in healthy tree



(Source: Pramod *et al.*, 2011a)

Fig. 8. Tangential longitudinal view of cambium in TPD-affected tree

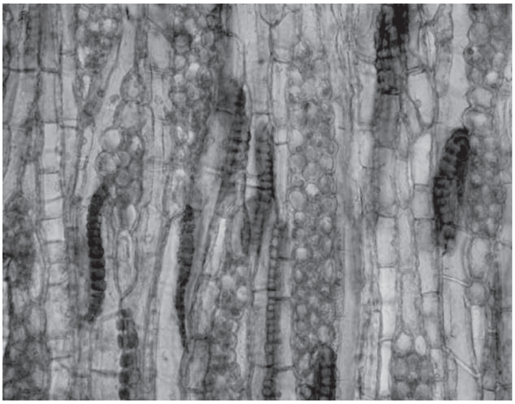
density of sieve tubes in soft bark of TPD affected trees over healthy trees was observed. Associated changes are the shortening of fusiform cambial cells, increase in number of cambial rays, number of terminal cells of ray, width of ray and number of cell layers constituting the cambial zone (Pramod *et al.*, 2011a).

The changes in the structure and activity of cambial cells might have a definite effect on the differentiation of phloem tissue which is responsible for radial and downward translocation of photosynthates, storage of metabolites and biosynthesis of latex. It has been reported that the level of endogenous ethylene produced as a result of tapping or the exogenous application of ethylene on the bark can enhance the incidence of TPD together with associated histological changes in the differentiation of cambial derivatives including latex vessels (Chrestin, 1989). The rhythm in the activity of cambium and the differentiation of laticifers from the derivatives in definite intervals are inevitable in a deciduous tropical tree like *Hevea* and the signals transmission for this rhythm are not fully understood. TPD will continue to remain as an irreversible phenomenon unless and until the rhythm of the cambium is brought back to its original form.

Definitive callose

Deposition of callose on the sieve plate and sealing the pores is a noticeable feature during inactivation of sieve tube. Two types of callose deposition in phloem of plants have been reported (Evert and Derr, 1964; Fahn,1982). The callose which initially deposits during the dormant phase of cambium and later disappears during the active phase is designated as dormancy callose. Due to aging or stress, the transport in sieve elements is blocked by the long-term deposition of callose which is termed as definitive callose. The definitive callose is generally considered as a wound polysaccharide consisting of β 1-3 glucan (Hori and Elbein, 1985). Deposition of definitive callose in the sieve tube of rubber trees under tapping was reported (Hebant and Fay, 1980).

Pramod *et al.* (2011b) studied the deposition of definitive callose with respect to TPD and stimulation in *H. brasiliensis* by using a number of histochemical stains. The information resolved on the deposition of callose on sieve plate varies with different staining procedures. Of the stains used, Congo red (Thompson and Hunt, 1966) was the fastest method with a staining time of 1-2 min. Tannic acid-ferric chloride-lacmoid (Cheadle *et al.*, 1953) requires longer staining duration but can resolve more information of sieve plate and sieve pores. Other stains used for sieve tubes are mild acid fuchsin and aniline blue (Johansen, 1940). Staining with O-dianisidine (Gopal and Thomas, 2012) gave deep brown colour for cell wall with more intensity for the sieve plate.



(Source: Pramod *et al.*, 2011b)

Fig. 9. Definitive callose deposition on the sieve plate of TPD-affected tree

The functionally active sieve elements in *Hevea* is confined to the inner part of the bark, with about 1.7 mm thickness adjacent to the cambial zone. The compound sieve plate in the bark of healthy trees is composed of several sieve areas, each with a number of aggregated pores, and each pore is lined by the deposition of a ring of callose. Outer region of the bark is termed hard bark where the sieve tubes are less active or inactive due to the thick deposition of callose on the sieve pores and the compound sieve plate appears as bead like structures in tangential sections of bark (Pramod *et al*., 2011a). In the TPD - affected panel of the tree, the open sieve pores are visible in the bark area measuring 0.2 mm from the cambial zone, whereas sieve pores in the unaffected area of the same tree were active up to 0.7 mm from the cambial zone. The non-functional sieve elements in the affected area were covered with thick deposition of definitive callose (Fig. 9). Measurements on sieve tubes such as density, length, sieve plate length, definitive callose deposition on the vertical and horizontal extent of sieve areas and the length of inter sieve area wall from both healthy trees and affected and unaffected areas of the TPD trees recorded drastic variation in the affected panel over healthy trees. The definitive callose deposition on vertical and horizontal extent of individual sieve areas in the TPD-affected bark is more than that found in the unaffected and in the healthy bark. Inactivation of sieve tubes even adjacent to the cambial zone was observed to be triggered by the thick deposition of definitive callose on the sieve plate when the TPD-affected bark was applied with ethephon or Vitex (a chemical formulation available in the market as remedy for TPD) (Pramod *et al*., 2011b). Intense sclerefication and a shift from the normal alignment of cells including sieve elements were also observed in the innermost part of the bark. The non-functional sieve elements in the TPD-affected bark gradually undergo lignification. Following cell wall lignification, the definitive callose starts to disappear and finally the entire sieve plate becomes fully lignified. From the results, it can be suggested that definitive callose deposition appears to be a starting point for beginning of the senescence process in sieve elements.

Plants develop different mechanisms to regulate solute transportation under stress conditions (Mengel and Kirkby, 1982; Meenakumari *et al*., 1998). Definitive callose deposition in the sieve areas is one of such defensive mechanisms, and as the intensity of stress increases, the extent of deposition also increases which is evident in the samples collected from TPD-affected trees and affected area applied with ethephon and Vitex. The callose production mediated by plasma membrane bound enzyme β 1, 3 glucan synthase in conjunction with precursors supplied by the endomembrane systems varies according to the degree of stress to the sieve elements (Evert, 1977; Cronshaw, 1981; Cumming and Taylor, 1990).

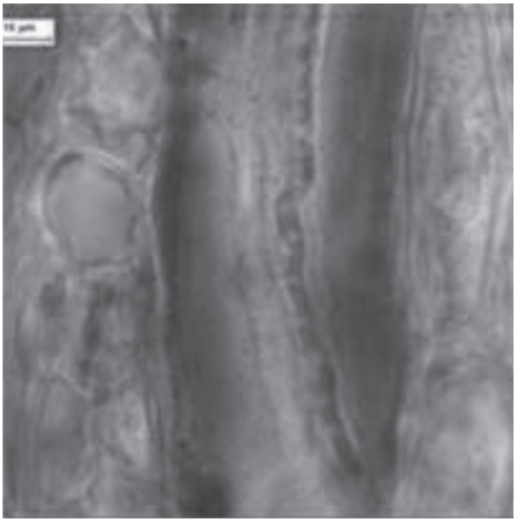
Phloem protein (P-protein)

Phloem protein occuring exclusively in the sieve tubes has been attributed to different functions that include mobilization of solute in the functionally active sieve tube elements and wound stress responses. It helps the plants to tide over adverse situations by agglomerating adjacent to the sieve plates in the sieve tube and regulating the movement of solutes (Esau *et al*., 1966; Parthasarathy, 1975). P-protein appears at sites of injury as an immediate wound response which is followed by deposition of definitive callose, lignin and suberin to seal off the wounded cells (Lipetz, 1970; Fahn, 1982; Thomas *et al*., 1995). On tapping, *Hevea* trees initiate certain defence mechanisms against the excess loss of photosynthates and cell organelles from the wounded tissue, and the formation of P-protein in the wounded sieve tube is one among them. Irrespective of age, both young stems and bark of mature trees of *Hevea* after wounding produce P-protein (Wu and Hao, 1987; 1990).

Radial and tangential longitudinal sections of the injured bark after tapping at a thickness of 40 µm were cut and stained with mercuric chloride-bromophenol blue for P-protein (Mazia *et al.,* 1953). The proteinaceous nature of the substance found in the sieve tube elements was confirmed by acid fuchsin staining (McCully, 1966).

The intensity of P-protein deposition varies under different stress situations. In the tapped trees of *Hevea,* P-protein appears as a narrow elongated structure in the sieve elements nearer to the sieve plate in the bark immediately next to the tapping cut. A thick plugging of P-protein appearing as a dense mass and covering most of the sieve plates (Fig. 10) was observed in the inner soft bark of TPD affected trees (Pramod *et al*., 2008). When 2.5 per cent ethephon was applied on the TPD-affected panel on rest, at fortnightly intervals for three months, the P-protein plugging was rare or disappeared as the sieve plates were covered by thick deposition of definitive callose.

TPD-affected trees when applied with Vitex become productive for a short period of time after which there was complete cessation of latex flow (Pramod, 2007; RRII, 2007). Bark samples collected at this stage showed certain structural and positional changes in P-protein in the sieve tube. The plug appeared a little away from the sieve plate on the opposite side of the plate. They were broad and elongated and covered almost complete length of the sieve plate and sometimes the plug penetrates into the sieve pore indicating a regulation of translocation in the sieve element. Such large mass of P-protein was mostly found in the sieve plate adjacent to the ray cell. The unproductive latex vessels in the TPD-affected region become active for a short while when applied with Vitex and the nourishments required



(Source: Pramod *et al.,* 2008)

Fig. 10. P-protein deposition near the sieve plate of TPD affected tree

for the latex biosynthesis might have been routed through alternative pathways like ray cells, as the sieve tubes remains non-functional.

The P-protein may appear in different structural forms with respect to ontogeny of sieve elements and degree of stress on the plant. The lipoid materials were found to be more in the TPD-affected tissues (Pramod, 2007) which may also result in increased P-protein plugging. Structural difference of the P-protein plug in the TPD-affected and unaffected area may be due to the differences in the metabolic activity of the element in relation to time at which they undergo maturation. The P-protein appearance is directly linked to the early stages of sieve element differentiation (Cronshaw, 1975). In TPD-affected regions of trees, the altered cambial activity may result in rapid differentiation of cambial derivatives (Thomas *et al*., 2006; Pramod *et al*., 2011a). The metabolic conditions of a rapidly differentiating cell may differ from its original rhythmic state, which may happen in the case of P-protein too.

Peroxidase activity

Sieve tubes, including the recently differentiated ones from the derivatives of cambium have been observed to get stained for peroxidase enzyme activity using both O-dianisidine and guaiacol (Gopal and Thomas, 2014). Tangential longitudinal sections of bark when stained with guaiacol showed variation in its staining pattern with respect to season. Cell walls of sieve tubes including sieve plates, and cytoplasm of phloic rays both in the soft and hard bark region showed positive indication of peroxidase activity with reddish brown colouration. Sieve tubes differentiated recently from the derivatives of cambium also stained deeply. Ray parenchyma in the soft bark of *H. brasiliensis* showed a different staining pattern as compared with that of other cells. The cell walls are not properly stained while the cytoplasmic contents acquire deep brick red color. The granulated contents suspended in the cytoplasm of the phloic rays showed streaming movement. Companion cells do not give any indication on peroxidase activity but these are found to be localized in the intercellular spaces of axial parenchyma cells in the soft bark.

Phloic rays exhibit seasonal variation for this enzyme while the activity is localized throughout the year in the sieve tubes. In the samples collected in December-January, phloic rays were unstained throughout the bark. Bark samples collected in the month of March and April showed deep coloration for phloic rays extending from the phellogen to the periphery of the cambial zone. In the month of June-July, the partially differentiated phloic rays in the cambial zone stained for peroxidase and subsequently differentiated ones were completely unstained. The phloic rays in the months of September and October (post monsoon period) remained unstained in the soft bark while it gave a brown colouration in the hard bark region. But the ray cells in the hard bark region neither possessed a deeply stained nature and the contents of the cytoplasm were not scrambled. The results implied that peroxidase activity showed variation in both radial and downward transport systems, and was influenced by climatic conditions.

Dynamic system in the sieve tube

Thomas *et al.* (2019) have reported the occurrence of a dynamic system in the sieve tubes of *H. brasiliensis* that evolve with both chaotic and linear display of patterns of globular objects with respect to time. Sieve tubes differentiated recently from the cambial zone in the tree trunk of *H. brasiliensis* showed uniform distribution of globular objects immersed in a viscous medium (Fig. 11). They were in a continuous state of motion, when 40-50 μm thick tangential longitudinal sections of both fresh and long term preserved bark were viewed under light microscope. The object measured an average diameter of 1.6 μm dispersed with a density of 200 per 1000 μm^2. The speed of the video-graphs of the moving objects recorded through the microscope was reduced to five per cent for convenience and found that these moving objects developed spatial patterns with respect to both loci and time within the sieve tube. At some point of time, patterns developing at different loci were different and were not repeating. Therefore, across the loci the displays were chaotic. In each locus, as the time proceeded, a linear pattern that lasted for 152 milliseconds developed, which was considered as a block. The pattern in a block remained static except at the 52nd and 102nd milliseconds in which two intermittent oscillating vibrations were observed.

In the TPD affected bark also, a similar kind of dynamic system existed in the sieve tubes. Two types of patterns for the block were observed and in one of the pattern, the duration of one block varied with an increment of 50 per cent time and such blocks were arranged alternatively (Thomas *et al.*, 2012b). In the other type, the duration of one block varied from the other by 100 per cent and in such cases the blocks did not develop any regular pattern for its orientation. The shortest duration of one block was recorded to be 76 milliseconds. The oscillating vibrations within a block varied from 4-5 and each with one millisecond for TPD-affected trees.

(Source: Thomas *et al.*, 2019)

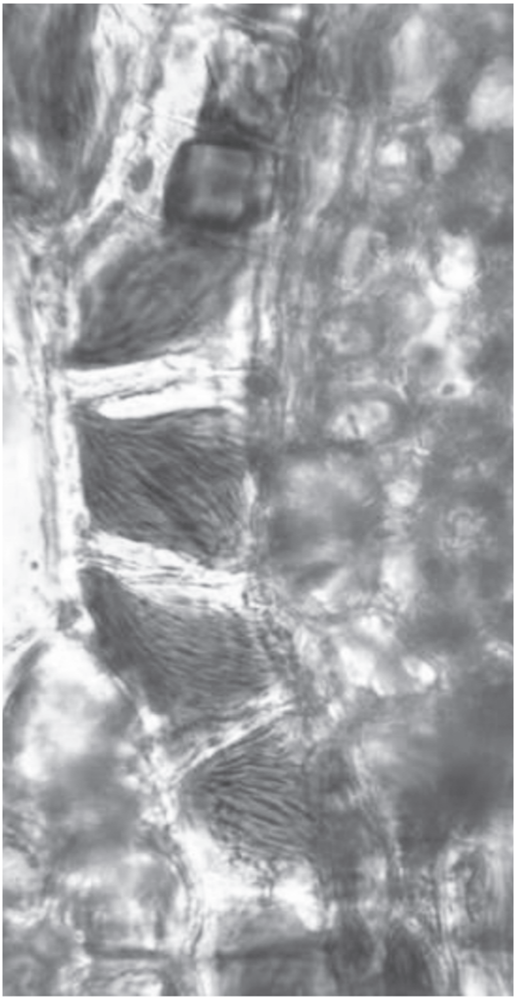
Fig. 11. Moving objects in the sieve tubes

The movement can be seen only in unprocessed sections of the bark and was not found in the serial microtome sections. While processing, the embedding media got impregnated into the specimen and caused disruption to both movement and stability of the particle (Thomas, 2012). A number of histochemical stains were tried but none of them could reveal the real chemistry of the moving object. This may be due to the displacement of objects in the dynamic system. The nature of movement for moving objects in the sieve tubes of higher plants was reported to exhibit 'brownian motion' or 'bouncing motion' as in the case with the starch granules released from the plastids or 'marker particles' in *Heracleum*

(Lee *et al.*, 1971). The particles in the sieve tube of *Hevea* are in a state of continuous and rhythmic motion developing spatial display of pattern which is unique. The reason why the rhythm has altered in TPD affected tree needs further investigations.

Protein Storing Cells (PSC)

The acute stress developed in the tapping panel of the TPD-affected trees resulted in many of the structural deformations such as permanent alteration in the rhythmic activity of cambium (Thomas *et al.*, 2006; Pramod *et al.*, 2011a), blocking of sieve tubes with definitive callose and P-protein (Pramod *et al.*, 2008, 2011b) and reduction in the dimensions of sieve tubes (Gopal and Thomas, 2012). The sieve tubes in the TPD-affected area are not in a position to mobilize photoassimilates in adequate quantity to the required site forcing the cambium to produce more alternative tissues like PSC (Thomas *et al.*, 2012a). Therefore, the density of sieve tubes was reduced considerably in the affected region. Protein storing cell, 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) are in close contact with the sieve elements of the secondary phloem, which is characterized by abundant fibrillar proteinaceous material of 67 kDa (Tian *et al.*, 1998). They are devoid of starch, lipid and tannin deposition. Functionally these cells are similar to that of companion cells with respect to the bidirectional movement of nourishments (Ghouse and Yunus, 1975). In healthy trees, the PSC are distributed in one or two layers surrounding the phloic rays (Fig. 12) and are also adjacent to the sieve tubes whereas its density was increased in TPD-affected trees (4 to 5 layers) as well as in TPD-affected regions of stimulated trees (Thomas *et al.*, 2012a). Dian *et al.* (1995) also noticed excessive formation of proteins in association with TPD to support the translocating tissue in the bark to remain active.



(Source: Thomas *et al.*, 2012a)

Fig. 12. Protein storing cells

In addition to the proteinaceous material in the PSC (67 kDa), plants possess 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 consisting of a dramatic increase in the level of 27 kDa and 45 kDa proteins and minor changes 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 affected trees of *Hevea* are different from VSP and may have some specific role when the tree is exposed to severe stress. The activity of cambium is an energy rich process and under stress condition, the tree develops its own mechanism to thrive over the unfavorable situation by strengthening other routes of translocation to compensate the acute shortage of assimilates (Sauter, 2000; Thomas *et al.*, 2012a).

As the downward translocation of photoassimilate through sieve elements in TPD trees was restricted due to definitive callose and P-protein, the plant mobilises available photoassimilates alternatively through radial transport system to the affected patch (Thomas *et al.*, 2013). Even though dimensional changes occurred for strengthening the radial transport system, phloic rays adhered with the formation of a layer of narrow elongated or bead like oval to oblong cells derived as a result of an entire or a part of fusiform initials. In general, the cambial initial that produced tissue responsible for downward translocation shifted its normal rhythm as a result of TPD and produced parenchymatous tissues with specific objectives of motivating metabolite mobilization.

Phytoplasma

Phytoplasma are microorganisms that occur in the plant cells, even in a healthy tissue but becomes evident only when the tissue or the system turned unhealthy either due to acute shortage of nourishment or under serious stress over a period of time (Esau, 1977; Musetti *et al.*, 2011). Phytoplasma can affect and retard the sieve tube activity by the deposition of P-protein or by the thick deposition of definitive callose (Buxa, 2014), and these two factors were evident in the sieve tubes of TPD-affected bark of *H. brasiliensis* and intensified when additional stress was exerted by the intensive application of ethephon on the bark tissue. Tree loss or complete drying of the bark is not evident as the minimum requirement of nourishment to the dry panel is being supplied through alternative mode such as a PSC or through the vascular rays with many alterations from its normal structure. The sieve tube in the dryness affected panel of *Hevea* is alive and further studies are needed to understand how long and to what extent the phytoplasma can survive.

Plant has its own mechanism to thrive situations that affect the normal growth or its existence. In the TPD affected panel, different defense mechanisms are employed in the sieve tubes to block/regulate its designated activity of downward translocation of photoassimilates. The blocking of this route leads to an acute shortage of metabolite availability to the latex vessels for latex biosynthesis. As a result of TPD, the associated structural deformations of sieve tubes in *H. brasiliensis* have got adequate attention.

CONCLUSION

Both morphological and anatomical abnormalities observed in the TPD-affected panel is the reflection of an altered activity of the cambium. The cambium in the affected panel functioning under acute starvation due to the insufficient supply of photoassimilate through the sieve tube tends to divide and differentiate the cambial derivatives, particularly fusiform initials in a disproportionate manner resulting in lowering the density of sieve tubes. Alternatively, more parenchymatous cells with specific functions are produced. The reason for the blocking of the sieve tube and a permanently altered activity of cambium in the affected panel needs to be further investigated.

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