2163

Annals of Botany **75**: 421–426, 1995

Anatomical and Histochemical Aspects of Bark Regeneration in *Hevea brasiliensis*

VINOTH THOMAS, D. PREMAKUMARI, C. P. REGHU, A. O. N. PANIKKAR and SARASWATHY AMMA, C.K.

Botany Division, Rubber Research Institute of India, Kottayam—686 009, Kerala, India

Received: 17 October 1994 Accepted: 9 January 1995

Natural rubber is obtained from the bark of *Hevea brasiliensis*. Both virgin and renewed bark are exploited for this purpose by a process known as tapping which involves controlled wounding and excision of bark tissues. The process of bark renewal and its nature and consequences thus assume importance. Anatomical and histochemical changes encountered with tapping were the deposition of lignin and suberin in the peripheral cells, enlargement of ray cells near the cut surface and the formation of a wound periderm. In the course of development, the wound phellogen made tangential continuity with the original phellogen in the virgin bark and functioned as a single phellogen. Vascular cambial activity was enhanced due to wound stimulus and the newly differentiated sieve tubes and ray cells were larger in size. The first periderm was functional for only a short period of time, after which a new meristematic zone developed in the inner tissues. Virgin and renewed bark differed in the proportion of soft and hard bark, amount and distribution of sclereids, tannin cells and crystals.

Key words: Bark renewal, *Hevea brasiliensis*, histochemistry, laticifers, para rubber tree, wound periderm.

INTRODUCTION

The para rubber tree [*Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell Arg.] is exploited commercially for latex, from which natural rubber is recovered. Latex is tapped over a period of about 25 years by systematic excision of the external tissue of the trunk, which contains the articulated branched laticifers (Bobilioff, 1923; George *et al.*, 1980; Hebant and Fay, 1980; Gomez, 1982; Rudall, 1987). On each tapping a thin shaving of the bark is removed leaving the cambium and a thin portion of the inner bark. The bark thus exposed but left unsevered is termed residual bark and is instrumental in the healing process, replacement of the lost tissues at the site of injury and progressive increase in thickness. The immediate effect of wounding is a shrinkage in the outermost cells of the bark on the wounded surface due to rupturing (Panikkar, 1974). These superficial cells undergo necrosis due to the deposition of lignin and suberin in order to check the entry of pathogens and the loss of water from the delicate interior tissues (Lipetz, 1970). Some of the hypodermal layers beneath the cut surface become enlarged and divide in irregular planes. The divisions become oriented parallel to the cut surface and this meristematic layer starts functioning as the phellogen in the process of bark regeneration.

The regeneration of bark is of high practical value in *H. brasiliensis* since the renewed bark is also exploited commercially for latex extraction in the usual cycle (Paardekooper, 1989; Premakumari and Panikkar, 1992). The present study on bark regeneration was carried out since there is a lack of detailed study regarding this aspect.

MATERIALS AND METHODS

The observations were made on trees of clone RRII 105 of *H. brasiliensis*. Five trees were randomly chosen from a clone trial laid out in 1979 and under exploitation since 1987 on a $\frac{1}{2}$S d/2 6d/7 (half spiral alternate daily tapping with 1 d tapping rest in a week) system of tapping, at the Central Experiment Station of the Rubber Research Institute of India (Chethackal, Pathanamthitta Dt., Kerala State, India). The first set of bark samples were collected on the day of tapping after cessation of latex flow. The observational trees were not tapped further to facilitate the study. Subsequent samples were collected at 1 d intervals up to day 4, 5 d intervals up to 30 d and at 15 d intervals up to 120 d of tapping. The last two sets of samples were collected after 150 and 180 d of tapping.

Samples were collected in such a way that they represented the virgin bark, the severed surface and the residual bark left uncut under different periods of regeneration. Samples were fixed in formalin-acetic acid (FAA) and paraffin blocks were prepared after dehydration through graded series of TBA (Johansen, 1940). Serial microtome sections of 8–10 μm were cut in three planes, i.e. transverse, tangential longitudinal and radial longitudinal and stained with Safranin 0–Fast Green FCF for general histology. The histochemical stains used were: periodic acid–Schiffs' reagent (Jensen, 1962) for total polysaccharides; phloroglucinol–HCl (Jensen, 1962) for lignin; tannic acid–ferric chloride (Johansen, 1940) for tannin; Aniline Blue (O'Brien and McCully, 1981) for callose and H_2SO_4 (Gahan, 1984) for suberin. Observations and photomicrographs were taken both under light and epi-fluorescence microscopy.

0305-7364/95/040421+06 $08.00/0

© 1995 Annals of Botany Company

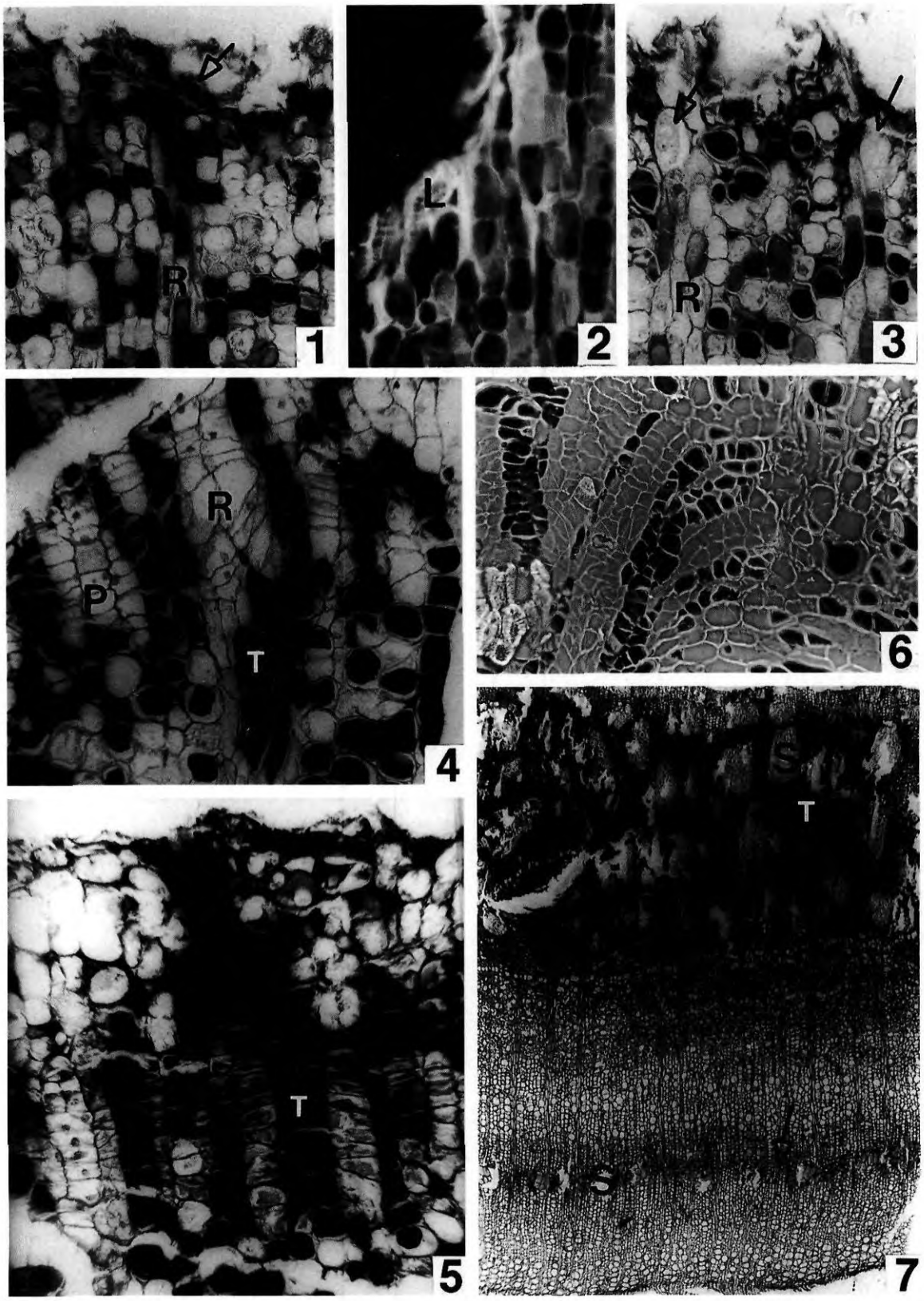


FIG. 1. Residual bark on the day of tapping. Arrow indicates wound cells at the cut surface. × 158. R, Ray cell.

FIG. 2. Fluorescent photomicrograph of lignified cells at the site of injury. × 158. L, Lignin.

FIG. 3. Enlarged ray cells (at arrows) below the wounded surface. × 158. R, Ray cell.

RESULTS

The wounding caused by tapping leaves the peripheral cells of the severed surface exposed. These cells on the residual bark soon become empty (Fig. 1) and necrotic. One day after tapping deposition of callose was observed in the perforations of the sieve elements adjacent to the wound. Suberin and lignin (Fig. 2) were found deposited in the intact cells mostly along the surface from day 2 onwards in a progressive manner and three to four such layers are formed. Lignification was noticed in all types of cells, including laticifers.

Near the cut end three to four layers of ray cells enlarged (Fig. 3), the maximum size being attained on day 3 of tapping. Enlargement of the ray cells was at right angles to its normal orientation (Fig. 4). Some of the parenchyma cells and ray cells at different loci, situated five to seven layers of cells beneath the cut surface, became meristematic. The cells were thin walled with prominent spherical nuclei at the centre. Cells divided periclinically or obliquely (Fig. 4) from day 3 onwards and day 4 up to four divisions were noticed in the ray cells. Dividing cells at different loci had the tendency to unite tangentially to form a more or less continuous wavy wound periderm, which was observable in the samples collected on day 10 after tapping (Fig. 5). Ray parenchyma and axial parenchyma (including tannin filled) were contributors to the formation of wound periderm. The wavy nature of the wound periderm was due to the irregular nature of the wound surface. Wound phellogen united with the original phellogen in the virgin bark and functioned more or less as a single meristematic zone. Wound periderm consisted of phellogen, phellem towards the exterior and phelloderm towards the interior, and the cells were regularly arranged in a radial direction (Fig. 5). The radial arrangement of the cells indicated the frequent tangential division of the phellogen. Some of the phellogen cells possessed tannin and the daughter cells produced were also observed to be filled with tannin (Figs 5 and 6). Phelloderm cells had cellulosic walls in the initial stage but in a later stage, many of them became lignified to form grouped sclereids (Fig. 7).

Wounding enhances cambial activity, which was evident from the bark samples collected 2–3 weeks after tapping. Cambial derivatives were produced in large numbers and could be seen as a distinct zone. In these cells the differentiation was initiated after about 1 week. Sieve tubes were more prominent due to their larger size than the remaining cells (Fig. 8). Newly differentiated sieve tubes in the regenerated bark had a larger diameter than those produced from the cambium in the uncut area. In the samples collected after 150 and 180 d of tapping, the number of undifferentiated cambial derivatives was minimal. The differentiated ray cells were thin walled and wider. Some of the parenchyma cells adjacent to the cambial zone possessed starch grains (Fig. 8). Laticifer initials could be found close to the sieve tubes (Fig. 8).

As the bark underwent regeneration more and more tissues were produced by both the cork and the vascular cambia. The original wound phellogen was functional only for a short period after which a new phellogen appeared in the soft bark (Fig. 7). This layer could be observed after a period of 4–5 months of tapping. In this case also, some of the parenchyma cells in the middle region of the soft bark became enlarged and meristematic and the dividing cells at different loci united to form a second periderm. The phellogen of this layer produced tissues centripetally and centrifugally as the previous one. Many of the phelloderm cells were lignified to form sclereids (Fig. 7). In samples collected from 5 month regenerated bark, the development of the deeper periderm was observed in the middle part of the soft bark, while in the virgin bark the second periderm was found in the soft bark quite away from the cambium. Because of the formation of an inner periderm, the tissues outside this layer became functionless. The first periderm was pushed to the periphery and later it developed shallow fissures and was sloughed off. The cells at the region where the bark peeled off were heavily suberized (Figs 9 and 10) and lignified. The amount of phelloderm was larger in the renewed bark when compared with the virgin bark.

Both vascular and cork cambia were involved in the regeneration of vertically wounded surfaces and the rate of renewal was more in comparison with the horizontally wounded area, where the major contribution was from the lateral cork cambium. In the horizontal area, cork cambium was less active and produced four to five layers of phellem, against 10–15 layers in the vertical surfaces (Fig. 11). In the region where vertical and horizontal cuts united, the cells were crushed and the normal orientation was disturbed (Fig. 11).

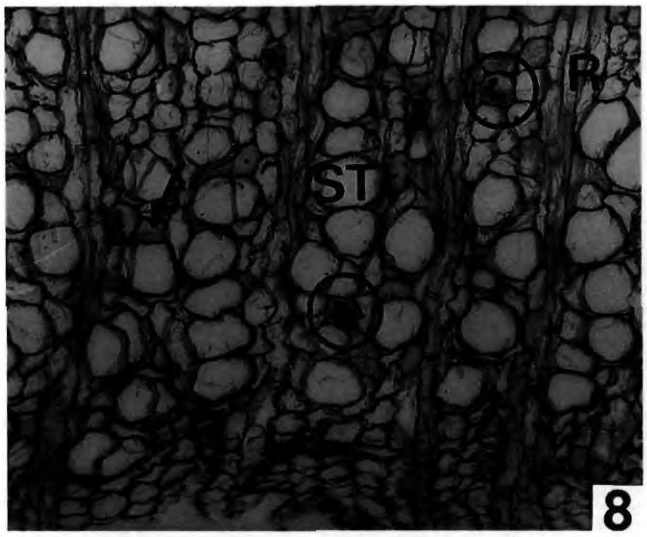
Virgin and renewed bark showed certain structural dissimilarities. The soft bark in the regenerated areas was thicker and the sclereids were delimited to the periphery. Groups of sclereids in the hard bark were irregularly arranged (Fig. 7) and its intensity was more in the renewed bark. Each group possessed 12–16 simple or branched pitted sclereids (Figs 7 and 9), of which many had tannin contents or crystals of rhomboid or druse types. Abundance of the sclereids disorganized phloem rays and increased the destruction of laticifer rings. Another notable aspect was the occurrence of tannin in cells, which were more in the outer region of the renewed bark. Tannin content was observed in the rays and axial parenchyma cells. In the virgin bark tannin cells were found scattered singly or in small groups (Fig. 1), mainly adjacent to the laticifers. Deposition of crystals also was greater in the vascular rays

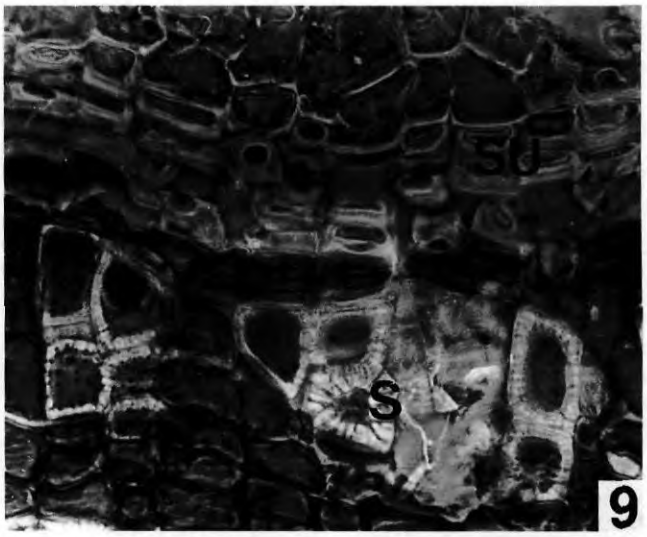
FIG. 4. Axial parenchyma and ray cells showing periclinal divisions. × 225. P, Axial parenchyma; R, ray cell; T, tannin cells.

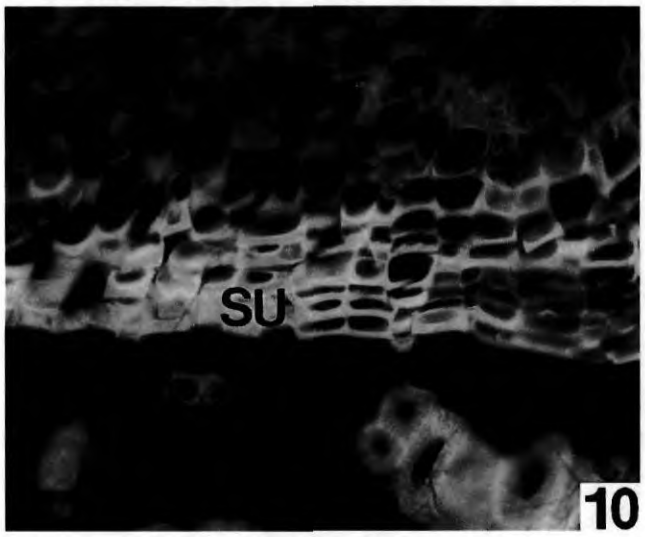
FIG. 5. Wound periderm. × 225. T, Tannin cells.

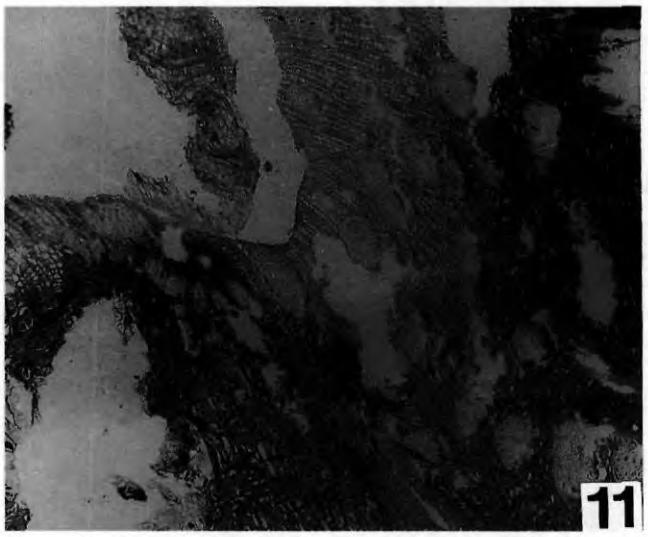
FIG. 6. Phellem with radially arranged tannin cells. × 158.

FIG. 7. Transection of regenerated bark showing hard and soft bark. Arrow indicates the formation of new periderm in the soft bark. × 10. S, Sclereid; T, Tannin cells.









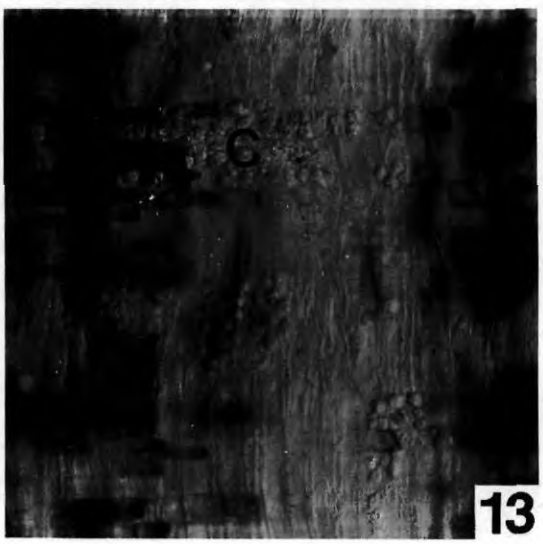


FIG. 8. Sieve tubes, laticifer (arrow) and starch grains (circles) in the regenerated bark. ×312. L, Laticifer; R, ray cell; ST, sieve tube.

FIG. 9. Phase contrasat photomicrograph of suberized cells of the cork tissue. ×312. S, Sclereid; SU, suberin.

FIG. 10. Autofluorescence of suberin in the cork. ×312. SU, suberin.

and parenchyma (Figs 12 and 13). In the renewed bark many of the cells adjacent to the laticifers possessed crystals.

DISCUSSION

The process of bark regeneration in *H. brasiliensis* is identical to that in other woody plants in that it involves the activity of both vascular cambium and phellogen: the continued activity of the vascular cambium and the formation and functioning of a new cork cambium (Lipetz, 1970; Rao, 1972). Morphologically wound healing can be of two types—deep and shallow. In *Hevea* it is the shallow type. A shallow wound is restricted to the destruction of inner bark tissue only and does not remove the vascular cambium, hence the cells in the soft bark will be contributors for wound periderm (Bostock and Stermer, 1989).

Wu and Hao (1991) have suggested that wound healing in *H. brasiliensis* has a close relation to bark regeneration since the injury to the bark leads to a series of anatomical and histochemical changes to the cells adjacent to the wound surface. It was evident by the formation of an impervious boundary as a result of the deposition of suberin and lignin in the extant cells, and the formation of a cork meristerm from extant tissues internal to the impervious boundary. Suberin and lignin were deposited in the wound cells in *Hevea*, peach and potato (Gomez, 1982; Biggs and Stobbs, 1985; Fay and Jacob, 1989; Borg-Oliver and Monties, 1993). But according to Wu and Hao (1991) no suberin deposition was found in the cells around the wound in *Hevea* bark. In the present study deposition of suberin in the wounded area of *Hevea* was confirmed with histochemical evidence. Hamzah and Gomez (1981) opined that the blocking of wound cells with suberin and lignin creates a favourable condition for the enhancement of phellogen activity. Formation of lignin and suberin in the wounded area is seen as a mechanism for resistance to degradation by micro-organisms (Vance, Kirk and Sherwood, 1980; Loebenstein, Spiegel and Gera, 1982) and as a barrier to moisture diffusion (Biggs and Stobbs, 1985).

In a wounded plant the physiological and biochemical activities are altered drastically. As a result of wounding, ethylene production has been increased in the parenchyma cells beneath the cut surface, leading to its dilatation growth either by cell enlargement and/or by cell division (Lipetz, 1970; Abeles, 1973; Yang and Hoffman, 1984; Lev-Yadun and Aloni, 1992). Vascular rays play an important role in the process of wound healing as there is enlargement of the ray at the site of injury and a predominant contribution to the wound phellogen formation in many plants (Sharples and Gunnery, 1933; Miller and Barnett, 1983). Lev-Yadun and Aloni (1990) described that phellogen is stimulated mainly by ethylene. They also mentioned that induction by ethylene and inhibition by auxin at physiological levels seem to regulate the polar patterns of periderm inhibition and formation.

Bark regeneration involves the replacement of new tissue at the site of injury which modifies its initial structure (Fay and Jacob, 1989). There was a difference in the proportion of soft and hard bark in the virgin and renewed bark of *Hevea*. According to the observations of Panikkar (1974) and Gomez (1983) renewed bark of *Hevea* has more soft bark. But Premakumari, Panikkar and George (1992) did not observe any significant difference between virgin and renewed bark for this trait. An increase in cell size of different tissues including rays, sieve tubes and laticifers of the renewed bark is in agreement with earlier findings (Premakumari, 1992). Another significant change was the amount and distribution of sclereids, tannin contents and crystals; those were more in the renewed bark, in accordance with the reports of Lipetz (1970) and Wu and Hao (1993).

According to Gomez (1982) and Fay and Jacob (1989) tapping was thought to activate the vascular cambium. Cambial derivatives will be produced in large numbers but the differentiation will be delayed, by which the gap between the first and second latex vessel rings near the cambium may vary (Hamzah and Gomez, 1981). According to Premakumari *et al.* (1992) laticifer diameter and intensity of anastomosing in the virgin and renewed bark were comparable. The two growth phases of bark varied significantly for the density of latex vessels.

The extent and duration of regeneration of bark varies with clones in *H. brasiliensis*. The extent of bark regeneration is also governed by various factors like depth of tapping, age of the tree, vigour of growth, agromanagement practices etc. (Edgar, 1958; Bostock and Stermer, 1989).

ACKNOWLEDGEMENT

We thank Dr M. R. Sethuraj, Director, Rubber Research Institute of India for encouragement and providing facilities.

LITERATURE CITED

Abeles FB. 1973. *Ethylene in plant biology*. New York: Academic Press.

Biggs AR, Stobbs LW. 1985. Fine structure of the suberized cell walls in the boundary zone and necrophylactic periderm in wounded peach bark. *Canadian Journal of Botany* **64**: 1606–1610.

Bobilioff W. 1923. *Anatomy and physiology of* Hevea brasiliensis. *Part I. Anatomy of* Hevea brasiliensis. Zurich: Art. Institut. Orell. Fussil.

Borg-Oliver O, Monties B. 1993. Lignin, suberin, phenolic acids and tyramine in the suberized, wound induced potato periderm. *Phytochemistry* **32**: 601–606.

Bostock RM, Stermer BA. 1989. Perspectives on wound healing in resistance to pathogens. *Annual Review of Phytopathology* **27**: 343–371.

Edgar AT. 1958. *Manual of rubber planting (Malaya)*. Kuala Lumpur: The Incorporated Society of Planters.

Fay de E, Jacob JL. 1989. Anatomical organization of laticiferous system in the bark. In: Auzac J, Jacob JL, Chrestin H, eds. *Physiology of rubber tree latex*. Boca Raton, Florida, USA: CRC Press, Inc.

Gahan PB. 1984. *Plant histochemistry and cytochemistry. An introduction*. London: Academic Press.

FIG. 11. Radial longitudinal section of renewed bark 6 months after tapping. ×8.

FIGS 12 and 13. Crystals (arrows) in the ray and axial parenchyma cells of renewed bark. Fig. 12, ×312; Fig. 13, ×158. C, Crystal.

George PJ, Premakumari D, Markose VC, Panikkar AON. 1980. The rubber tree (*Hevea brasiliensis* Muell. Arg.) In: Radhakrishna Pillai PN, ed. *Handbook of natural rubber production in India.* Kottayam: The Rubber Board.

Gomez JB. 1982. *Anatomy of* Hevea *and its influence on latex production.* MRRDB Monograph No. 7, Kuala Lumpur.

Gomez JB. 1983. *Physiology of latex (rubber) production.* MRRDB Monograph No. 8, Kuala Lumpur.

Hamzah SB, Gomez JB. 1981. Anatomy of bark renewal in normal puncture tapped trees. *Journal of the Rubber Research Institute of Malaysia* **29**: 86–95.

Hebant C, Fay de E. 1980. Functional organisation of the bark of *Hevea brasiliensis* (rubber tree): a structural and histoenzymological study. *Zeitschrift für Pflanzenphysiologie* **97**: 391–398.

Jensen WA. 1962. *Botanical histochemistry. Principles and practice.* San Francisco: W. H. Freeman and Co.

Johansen DA. 1940. *Plant microtechnique.* New York: McGraw-Hill Book Co.

Lev-Yadan S, Aloni R. 1990. Polar patterns of periderm ontogeny, their relationship to leaves and buds, and the control of cork formation. *IAWA Bulletin* **11**: 289–300.

Lev-Yadun S, Aloni R. 1992. Experimental induction of dilatation meristems in *Melia azedarach.* L. *Annals of Botany* **70**: 379–386.

Lipetz J. 1970. Wound healing in higher plants. *International Review of Cytology* **27**: 1–28.

Loebsenstein G, Spiegel S, Gera A. 1982. Localized resistance and barrier substances. In: Wood RKS, ed. *Active defence mechanisms in plants.* London: Plenum Press.

Miller H, Barnett JR. 1983. The formation of callus at the graft interface in Sitka Spruce. *IAWA Journal* **14**: 13–21.

O'Brien TP, McCully ME. 1981. *The study of plant structure: principles and selected methods.* Melborune: Australia Termarcarphi Pvt. Ltd.

Paardekooper EC. 1989. Exploitation of the rubber tree. In: Webster CC, Baulkwill WJ, eds. *Rubber.* London: Longman Scientific & Technical.

Panikkar AON. 1974. *Anatomical studies on* Hevea brasiliensis *Muell. Arg. with special reference to bark regeneration.* Ph.D. Thesis, Birla Institute of Technology & Science, Pilani, India.

Premakumari D. 1992. *Variability, correlations and path coefficient analysis for yield in relation to anatomical characters in* Hevea brasiliensis *(Willd. ex Adr. de Juss.) Muell Arg.* Ph.D. Thesis. University of Kerala, Trivandrum, India.

Premakumari D, Panikkar AON. 1992. Anatomy and ultracytology of latex vessels. In: Sethuraj MR, Mathew NM, eds. *Natural rubber: biology, cultivation and technology.* Amsterdam: Elsevier.

Premakumari D, Panikkar AON, George PJ. 1992. Comparative anatomy of virgin and renewed bark in *Hevea. Journal of Plantation Crops* **20**: 204–206.

Rao AN. 1972. Periodic changes in the cambial activity of *Hevea brasiliensis. The Journal of the Indian Botanical Society* **51**: 13–17.

Rudall P. 1987. Laticifers in Euphorbiaceae: a conspectus. *Botanical Journal of the Linnean Society* **94**: 143–163.

Sharples A, Gunnery H. 1933. Callus formation in *Hibiscus rosa-sinensis* and *Hevea brasiliensis.* Muell. Arg. *Annals of Botany* **47**: 829–836.

Vance CP, Kirk TK, Sherwood RT. 1980. Lignification as a mechanism of disease resistance. *Annual Review of Phytopathology* **18**: 259–288.

Wu J, Hao B. 1991. Effect of ethephon on bark wound healing in *Hevea brasiliensis. Indian Journal of Natural Rubber Research* **4**: 91–96.

Wu J, Hao B. 1993. Ultrastructure of *Hevea* bark on tapping: parenchyma cells in secondary phloem. *Journal of the Rubber Research Institute of Malaysia* **29**: 86–195.

Yang SF, Hoffman NE. 1984. Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology* **35**: 155–189.