MICROMORPHOLOGY OF THE LEAVES OF *HEVEA BRASILIENSIS* AND *H. BENTHAMIANA*

Vinoth Thomas and C.K.Saraswathyamma
Rubber Research Institute of India, Kottayam 686 009, Kerala, India.

Submitted: 26 August 2002 Accepted: 30 December 2004

Thomas, V. and Saraswathyamma, C.K. (2004). Micromorphology of the leaves of *Hevea brasiliensis* and *H. benthamiana*. *Natural Rubber Research*, **17**(2) : 126-132.

Light and scanning electron microscopic studies of the leaf surface of *Hevea brasiliensis* and *H. benthamiana* revealed that both the surfaces of the leaf lamina are sheathed in thick ornamental cuticle, which is more marked on the abaxial surface. The cuticular ornamentation of both the species appears as a reticulum of buttressed ridges around the stomata on the abaxial surface. The stomatal apparatus appears like a crypt. The buttressed ridges are sculptured with small cuticular continuities that appear as hairy appendages. In *H. benthamiana* the ridges are thick and arranged more or less compactly reducing the width of the stomatal aperture. The cuticle over the laminar nectary is thick and smooth. The cuticular pattern on the adaxial lamina is more or less uniform with long parallel striae arranged closely. The thick cuticle is covered with a thin layer of epicuticular wax. Although different interpretations have been given for the surface sculptures of *H. brasiliensis*, the present study shows that the important component responsible for the leaf features is cuticular in nature. The possible role of cuticle and epicuticular wax in disease resistance and ecological adaptations of *Hevea* is suggested.

Key words: Cuticular ornamentation, Epicuticular wax, Extrafloral nectary, *Hevea benthamiana*, *Hevea brasiliensis*, Leaf micromorphology.

INTRODUCTION

Hevea brasiliensis, the prime source of natural rubber, is a deciduous tropical tree having trifoliate compound leaves with dorsiventral leaflet laminae. The leaf surface of *H. brasiliensis* is highly ornamented and different interpretations have been given for its origin *viz.*, ridge like thickenings of the cell membrane (Bobilioff, 1923), cuticular striations (Rao, 1963; Martin and Juniper, 1970; Sanier and d'Auzac, 1986) and epicuticular wax (Gomez, 1982; Gomes and Kozlowski, 1988; Premakumari *et al.*, 1989; Premakumari and Saraswathyamma, 2000). Rao *et al.* (1988) estimated the amount of epicuticular wax present in different clones of *Hevea* and suggested that it is an ecological adaptation. Its role against the invasion of pathogens was suggested by Premakumari *et al.* (1989). The causative organism of the leaf borne diseases of rubber enter into the plant system as fungal hyphae, either through the stomatal opening or by piercing the cuticle. *H. brasiliensis* is more susceptible to leaf diseases than *H. benthamiana*, which has thick and leathery leaves. The significance of cuticular striations and epicuticular wax in regulating the size of the stomatal pore making it difficult for the entry of pathogens is well documented in many plant species including *Hevea* (Martin and Juniper, 1970; Premakumari and Saraswathyamma, 2000). The present attempt is aimed at elucidating the leaf mi-

Correspondence: Vinoth Thomas (email: rrii@vsnl.com; vt@rubberboard.org.in)

cromorphology of *Hevea brasiliensis* and *H. benthamiana,* and discussing its significance in disease resistance and ecological adaptations.

MATERIALS AND METHODS

Mature leaves of *H. brasiliensis* (Willd ex Adr. de Juss.) Muell. Arg. (clone RRII 105) and *H. benthamiana* Muell. Arg. of family Euphorbiaceae, were collected and fixed in formaldehyde-acetic acid- ethyl alcohol mixture (Johansen, 1940). Leaf samples were dehydrated through graded series of tertiary butyl alcohol, followed by impregnation with Tissue Prep wax (52.5 °C) in which they were finally embedded. Serial microtome sections of 6 to 8 µm were cut, deparafinized through xylene-alcohol series, and stained with toluidine blue O (O'Brien and McCully, 1981) and periodic acid-Schiff's reagent (Jensen, 1962). Epidermal peels were taken from both mature and dry leaves by boiling the samples in nitric acid (60%) and potassium chlorate (Johansen, 1940). For semi-thin sections, leaf samples were fixed overnight in gluteraldehyde and post fixed in OsO_4. Dehydration was carried out in graded acetone series and embedding was done in Spurr's resin (Spurr, 1969). Sections of 1µm thickness were cut on LKB ultramicrotome (at CPCRI, Kayamkulam) and stained with Methylene blue- azur II –basic fuchsin (Humphrey and Pittman, 1974). Observations were made and photomicroghaphs were taken using a Leica Diaplan fluorescent microscope.

For scanning electron microscopy, fresh and processed samples were used. Processing was by deparafinisation followed by dipping in chloroform for 30 seconds. The samples were coated with Au after dehydration and were observed under Hitachi H 6010A SEM (at CMFRI, Cochin).

RESULTS AND DISCUSSION

The dorsiventral leaflet of *Hevea* has an upper and a lower epidermis, composed of rectangular cells. Stomata are confined to the abaxial surface of the lamina and are slightly sunken from the level of the epidermal cells (Figs.1 & 2). Both the epidermal layers are sheathed in cuticle, which forms a continuous membrane sculptured with prominent striations. These striations are most marked on the abaxial surface of the leaf lamina (Figs. 3, 4 & 5). The cuticular ornamentation in both the species of *Hevea* appears as a reticulum of buttressed ridges around the stomata and the stomatal apparatus looks like a stomatal crypt. From the ridged striations, arise small cuticular continuities, which appear as hairy appendages as reported by Rao (1963) for the leaves of *H. brasiliensis.* The ridges on the leaves of both the species are prominent and distinct from one another leaving comparatively less thickened area between the ridges. They are thick and more or less compactly arranged in *H. benthamiana* (Fig. 5) whereas in *H. brasiliensis* they are slender with tail like extensions and are not arranged closely. *H. benthamiana* possesses uniseriate hairs that arise from the midrib, veins and lamina on the abaxial side of the leaf (Fig. 6) as reported by Wycherley (1992). Cuticular sculpture on the midrib and veins are different from that on the rest of the lamina. In the midrib region the cuticle appears morphologically as small elongated ridges and furrows. A transection of the leaf through the midrib showed that the outer walls of the epider-

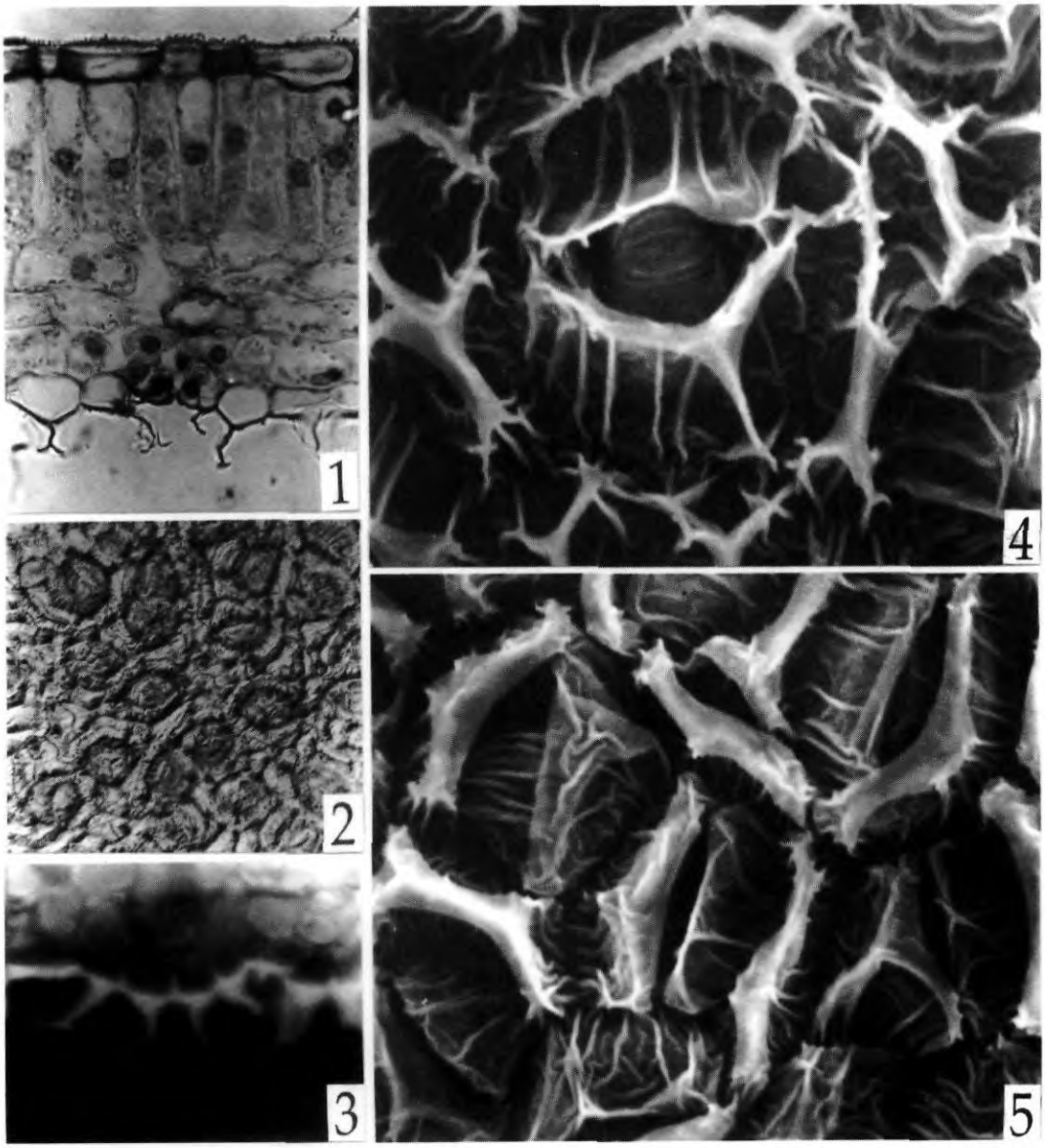

Fig. 1. Transection of the leaf of *H. brasiliensis* showing thick cuticle on both sides of the lamina (X160)
Fig. 2. Reticulate pattern of the cuticle (X160)
Fig. 3. Cuticular extentions of *H. benthamiana* under epifluorescence (X320)
Figs.4 & 5. Leaf ornamentation of *H. brasiliensis* and *H. benthamiana* respectively under SEM (X750 and X500)

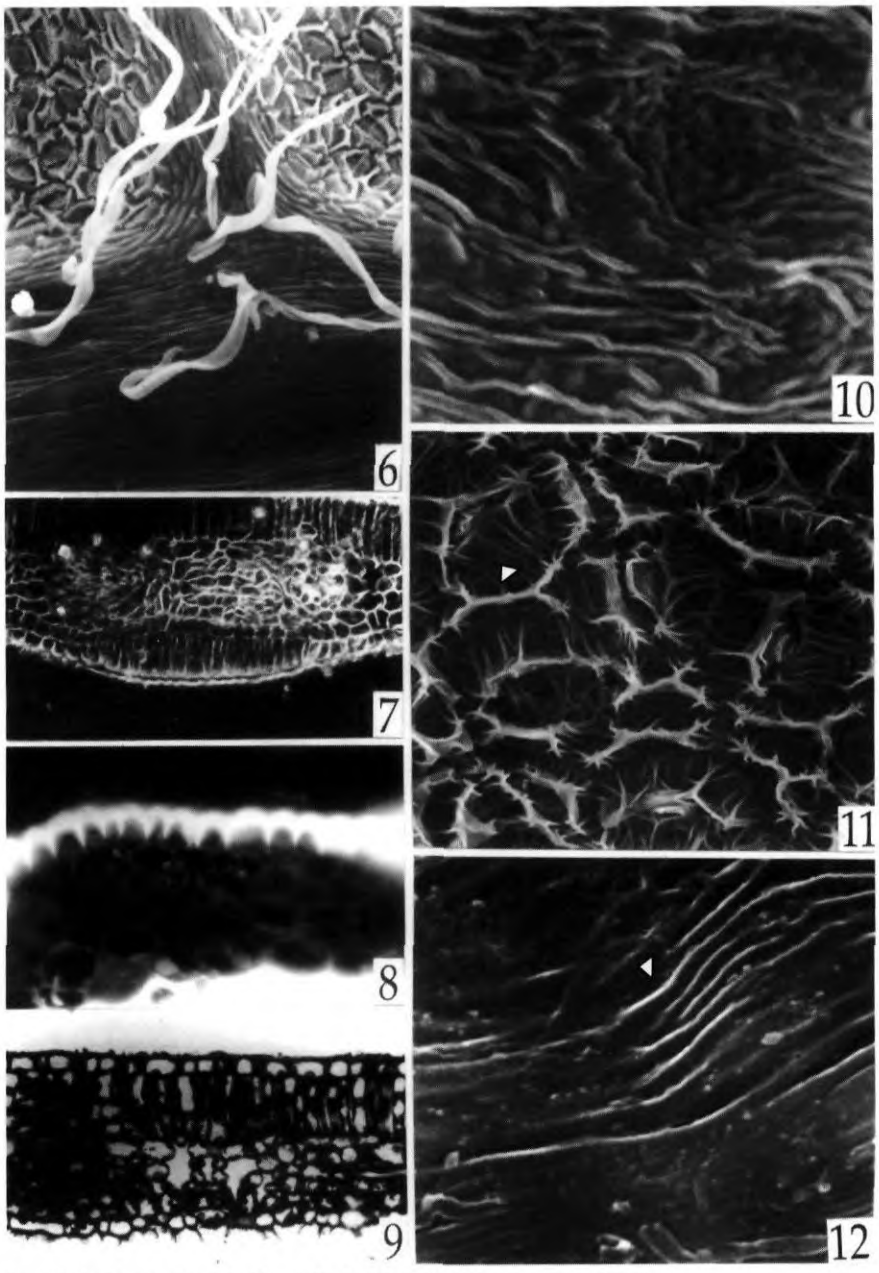

Fig. 6. Uniseriate hairs on abaxial side of the lamina of *H. benthamiana* (X500)
Fig. 7. Laminar nectary of *H. brasiliensis* ensheathed by thick cuticle (at arrow head) (X85)
Fig. 8. Fluorescence of cuticle on the adaxial side of *H. benthamiana* (X320)
Fig. 9. Transection of the leaf of *H. brasiliensis* taken after treatment with OsO_4 (X60)
Fig. 10. Adaxial laminar surface of *H. brasiliensis* under SEM (X800)
Figs. 11 & 12. Deparafinized cuticle on the abaxial and adaxial sides of *H. brasiliensis* leaf showing cuticle (at arrow head) (X400; X1250)

mal cells in this region are round and covered externally with a thick cuticle. The cuticle on the midrib and vein are intruded into the epidermal cells (Fig. 8). The cuticular pattern on the adaxial laminar surface of both the species is a more or less uniform distribution of long parallel striae arranged closely (Fig. 10). In both the species, hairs and stomata were not observed on the upper epidermis.

The compactly arranged thick buttressed reticulum of cuticular ornamentation in *H. benthamiana* contributes to the leathery nature of the leaf and at the same time provides protection from excessive stomatal transpiration and the invasion of pathogens. *H. benthamiana* is generally considered as less susceptible to most of the leaf diseases, particularly to abnormal leaf fall disease caused by *Phytophthora* sp. Due to this, it is recommended for crown modification of high yielding but susceptible clones of *H. brasiliensis*, in regions where *Phytophthora* disease is prevalent (Pillai *et al.*, 1989). The stiff and leathery nature of the leaf followed by the deposition of cell wall material also tends to reduce net photosynthesis (Dale and Milthorpe, 1983).

Apart from the occurrence of functional extrafloral nectaries at the petiole-petiolule juncture, *H. brasiliensis* possesses functional nectaries on the lamina too (Thankamma and George, 1968). The laminar nectaries are confined to the abaxial surface and are circular in appearance, covered with a thick cuticle (Fig. 7) devoid of cuticular appendages. The nectary consists of a row of radially elongated epidermal and subepidermal cells. The cuticle on the nectary is thick and intrudes into the nectariferous cells. Stomata and pores are not observed on the surface of these raised nectaries (Thankamma and George, 1968).

Among the most important fatty substances i.e., cutin, suberin and waxes, present in the leaf epidermal cell, wax is an important constituent of the cuticle, embedded within and sometimes exuded over the surface of the cuticle as epicuticular wax. The wax may be deposited on the lamina in large or small flakes or granules, rods, tubes or sheets (Martin and Juniper, 1970). The waxes melt readily and can easily be extracted by fat solvents, whereas cutin and suberin do not melt and are not readily soluble in fat solvents (Esau, 1965). Attempts have been made earlier to understand the structure of cuticle after removal of surface wax with organic solvents (Wattendorff and Holloway, 1982). When abaxial and adaxial leaf surfaces of both the species were deparafinized and viewed under SEM, the buttressed ridges, hairy appendages and long parallel striae were observed as leaf sculptures (Figs.11 & 12). Reticulate pattern and hairy appendages on leaf surface could also be observed in epidermal peels from the leaves boiled in 60% HNO_3 (Fig.2), in serial microtome sections of leaf after deparafinisation in xylene (Fig.1), brightened cuticle in sections on both the sides of the lamina under epifluorescence (Figs. 3 & 8), and thin (1 µm) sections of leaf fixed in OsO_4 (Fig.9). This indicated that the surface ornamentations are basically cuticular extensions rather than epicuticular wax.

This confirms that the sculptures on the leaf lamina of both *H. brasiliensis* and *H. benthamiana* are predominantly cuticular ornamentations. This observation is in agreement with the earlier observations on *Hevea* by Bobilioff (1923) and Rao (1963) that the

laminar ornamentations are cuticular striations. Martin and Juniper (1970) also observed that laminar cuticle is thick and covered by comparatively little surface wax. Among the four different types of waxy coverings described by de Bary (1884), the wax on *Hevea* leaf is in the form of a continuous film or encrustation covering the whole cuticle, leaving the stomatal openings, like a brittle transparent glaze.

It has been reported that the abaxial side of the mature leaf has parallelly arranged tubular striations formed due to the elongation and anastomosing of the striae, and that the sunken stomata are almost completely covered by wax coating (Premakumari *et al.*, 1989). Anastomosing of the cuticular striae and deposition of wax on the stomatal opening of the mature leaf could not be observed in spite of adopting different techniques in the present study. The cuticle is constituted of compounds like cutin and suberin, which are polymerised compounds of fatty acids that become rigid on deposition (Romberger *et al.*, 1993). In each plant the cuticular pattern is distinct and does not change with age. The cuticular pattern often preserves many of the characteristic features of the underlying epidermis such as stomata and hairs, and their distribution (Ahmad, 1975; Cutter, 1978). Based on the study conducted with *Hevea* clones grown under different climatic conditions, Gomez (1982) reported that the reticulate pattern on the leaf surface is not affected by environmental factors. Complete closure of the stomata by the deposition of wax is not expected, as it would affect gas exchange leading to the nonfunctioning of the leaf. Such a phenomenon has not been observed even in plants grown under adverse climatic conditions. The development of wax is affected by light, being more under high light intensities. The leaf of *Hevea* being dorsiventral, only the wax on the adaxial surface may contribute to the reflection of excess light. The wax present on the abaxial side may reduce cuticular transpiration and increase resistance to leaf diseases.

The occurrence of epicuticular wax on the leaves of different clones of *Hevea brasiliensis* was estimated by Rao *et al.* (1988) for relating it with drought tolerance. Holloway (1982) suggested that subcuticular wax is also an important factor enabling the plants to withstand dry habitats. When epicuticular wax is deposited on a wavy thick cuticle, the amount of wax deposited per unit area of the lamina is more. Such wax deposition may reduce cuticular transpiration and reflect excess light thus serving as an ecological adaptation. Presumably, this may be one of the reasons why *H. brasiliensis* grows well even in the drought - prone areas in India.

ACKNOWLEDGEMENT

Authors thank Dr. N.M. Mathew, Director, Rubber Research Institute of India for his support and encouragement.

REFERENCE

Ahmad, K.J. (1975). Cuticular studies in some species of *Lepidagathis* and B*arleria*. *Botanical Gazette*, **136** :129-135.

de Bary, A. (1884). Comparative anatomy of the vegetative organs of the phanerogams and ferns. Transl. Bower and Scott, Oxford University Press, London.

Bobilioff, W. (1923). Anatomy and physiology of *Hevea brasiliensis*. Art Institut Orell Fussli, Zurich, 150p.

Cutter, E.G. (1978). Plant anatomy. Ed, 2, Edward Arnold, London.

Dale, J.E. and Milthorpe, F.L. (1983). General features of the production and growth of leaves. In: *The Growth and Functioning of Leaves*. (Eds. J.E. Dale and F.L. Milthorpe), Cambridge University Press, London. pp.151-178.

Esau, K. (1965). Plant anatomy. Ed. 2, John Wiley & Sons Inc., New York, 767p.

Gomes, A.R.S. and Kozlowski, T.T. (1988). Stomatal characteristics, leaf waxes and transpiration rates of *Theobroma cacao* and *Hevea brasiliensis* seedlings. *Annals of Botany*, **61** : 425-432.

Gomez, J.B. (1982). Anatomy of *Hevea* and its influence on latex production. Malaysian Rubber Research and Development Board, Kuala Lumpur, 75p.

Holloway, P.J. (1982). Structure and histochemistry of plant cuticular membranes: An overview. In: *The Plant Cuticle*. (Ed. D.F. Cutler, K.L. Alvin, and C.E. Price). Academic Press, London.

Humphrey, C.D. and Pittman, E.F. (1974). A simple methylene blue- azure II: Basic fuchsin stain for epoxy- embedded tissue sections. *Stain Technology*, **49** : 9-14.

Jensen, W.A. (1962). Botanical histochemistry. W.H. Freeman & Co., San Fransisco.

Johansen, D.A. (1940). Plant microtechnique. McGraw Hill Book Co., New York.

Martin, J.T. and Juniper, B.E. (1970). The cuticles of plants. Edward Arnold (Publishers) Ltd., Edinburgh, 347p.

O'Brien, T.P. and McCully, M.E. (1981). The study of plant structure: principles and selected methods. Australian Termarcarphi Pvt. Ltd., Melborune.

Pillai, P.N.R., Krishnankutty, V. and Edathil, T.T. (1989). Crown budding: A method to reduce cost of production of natural rubber in India. *Journal of Plantation Crops*, **16** (Supplement): 277-279.

Premakumari, D., Panikkar, A.O.N. and Sethuraj, M.R. (1989). Development of epicuticular wax and cuticular ornamentation in *Hevea brasiliensis* (Willd.ex Adr. de Juss.) Muell. Arg. *Indian Journal of Natural Rubber Research*, **2** : 55-60.

Premakumari, D. and Saraswathyamma, C.K. (2000). The para rubber tree. In: *Natural Rubber: Agromanagement and Crop Processing* (Eds. P.J. George and C. Kuruvilla Jacob), Rubber Research Institute of India, Kottayam, India, pp.29-35.

Rao, A.N. (1963). Reticulate cuticle on leaf epidermis in *Hevea brasiliensis* Muell. Arg. *Nature*, **197** : 1125.

Rao, G.G., Devakumar, A.S., Rajagopal, R., Annamma Y., Vijayakumar, K.R. and Sethuraj, M.R. (1988). Clonal variation in epicuticular waxes and reflectance: Possible role in drought tolerance in *Hevea*. *Indian Journal of Natural Rubber Research*, **1** : 84-97.

Romberger, J.A., Hejnowicz, Z. and Hill, J.F. (1993). Plant structure: Function and development. Springer – Verlag, Berlin, 524p.

Sanier, C. and d'Auzac, J. (1986). Etude anatomique de l'epiderme de la feuille d'*Hevea brasiliensis* Kunth. (Mull. Arg.). *Compte Rendudu Academi Science, Paris*, **303** : 325-330.

Spurr, A.R. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research*, **26** : 31-43.

Thankamma, L. and George, K.V. (1968). Extrafloral nectaries in *Hevea brasiliensis*. *Rubber Board Bulletin*, **9** : 41-46.

Wattendorff, J. and Holloway, P.J. (1982). Studies on the ultrastructure and histochemistry of plant cuticles: Isolated cuticular membrane preparations of *Agave americana* L. and the effects of various extraction procedures. *Annals of Botany*, **49** : 769-804.

Wycherley, P.R. (1992). The genus *Hevea*: Botanical aspects. In: *Natural Rubber: Biology, Cultivation and Technology* (Eds. M.R. Sethuraj and N.M. Mathew). Elsevier, Amsterdam, pp.50-66.