Indian J. Nat. Rubb. Res. 2 (1): 55–60, 1989

DEVELOPMENT OF EPICUTICULAR WAX AND CUTICULAR ORNAMENTATION IN *HEVEA BRASILIENSIS* (WILLD. EX ADR. DE JUSS.) MUELL. ARG.

D. Premakumari, A. O. N. Panikkar and M. R. Sethuraj

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 J. Nat. Rubb. Res. 2(1): 55–60

The general pattern and phenology of epicuticular wax in *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. was studied with the aid of scanning electron microscope. The developmental stages of wax formation in relation to the phenology of leaf in *Hevea* and general pattern of epicuticular wax on the leaf surface, petiole, petiolule, tender stem and fruit wall have been described. Functional importance and possible utility of the wax pattern in disease management and clone identification in *Hevea* have been discussed. The role of epidermal structure on organographic specificity to *Phytophthora* leaf fall disease in *Hevea* has been confirmed on the basis of wax pattern.

Key words — Cuticular ornamentation, Epicuticular wax, Epidermis, *Hevea brasiliensis*.

D. Premakumari (for correspondence), A. O. N. Panikkar and M. R. Sethuraj, Rubber Research Institute of India, Kottayam – 686 009, India.

INTRODUCTION

Surface ornamentation of cuticle mainly consists of striae which are ridges or folds of epicuticular waxes. Deposition of epicuticular wax is related to reduced rate of epicuticular transpiration (Clark and Levitt, 1956), resistance to leaf diseases and application of insecticides and fungicides on plants. The architecture of wax is important in the ability of the plant to reduce cuticular transpiration (Chambers and Possingham, 1963). In *Hevea* the leaves are compound and dorsiventral, having reticulate ornamentation on the abaxial side (Rao, 1963). In the present study the developmental stages of wax formation on the abaxial surface of leaflets, organographic variability in cuticular architecture and possible functional significance are discussed.

MATERIALS AND METHODS

Basal leaves representing different growth stages from bud emergence stage to hardened stage, as arbitrarily classified (Anon, 1976), as well as fully hardened leaves were collected. The samples thus covered bud-break stage (bud emerged and grown to a length of just two cm), leaflet stage (the leaves of the terminal flush still expanding and copper to reddish in colour), pendant stage (the leaves almost completely expanded and green but still limp), hardened stage (the leaves of the terminal flush fully expanded and just hardened, lamina in proper position) and fully hardened stage.

Fresh leaf pieces from the central position of the middle leaflet and samples from petiole, petiolule, tender green stem and the pericarp of mature green fruits, belonging to clone Tjir 1, were collected. Specimen conductivity was improved by painting the edges of the leaf tissue against the stub with conductive silver paint (Silver Print, G. C. Electronics, Rodeford, Illinois) and then uniformly coating the specimen with gold: palladium (60:40) alloy using the Fine Coat Ion Sputter JFC 1100, for three minutes.

RESULTS

Even at the bud break stage wax formation has been found started. At this stage the waxy coating over the lower epidermis is only a wavy layer made of folds of wax (Plate 1.1). Thick ridges of wax, median in position and oriented parallel to the long axis of the epidermal cell producing thin and small filamentous striae, were noted at the leaflet stage (Plate 1.2). At the pendant stage the ridges became more prominent. The occurrence of numerous filamentous arms, still elongated and with tapering ends, was clearly exhibiting the reticulate appearance (Plate 1.3).

When the leaves get just hardened, the ridges as well as the striae became thicker and prominent. Both were observed to be of more or less the same prominence and were clear at the same focus, while the epidermal cells were out of focus indicating that the stomata were at a lower level than the epicuticular wax layer and were partly covered by wax. It is at this stage, the cuticular ornamentation shows a reticulum of buttressed ridges over the epidermis partly overlapping the stomata (Plate 1.4). The stomatal apparatus at this stage looks like a stomatal crypt (Plate 2.3).

It was observed that the growth and thickening of the striae continued further with the hardening of the leaves and get anastomosed resulting in the formation of very long parallely arranged striae in continuous layers covering the epidermis. At this growth stage of the lamina, the stomata were completely sunken and covered (Plate 1.5).

The upper epidermis is more or less glaucous owing to an even distribution of vermiculate striae closely entangled and without buttresses (Plate 2.2).

Epicuticular pattern of the fruit wall is entirely different from that of the leaf blade. The waxy layer was generally more thick. However, this layer was of uneven thickness, looking like an unevenly distributed group of clouds (Plate 2.1) with interruptions at the portions of stomatal openings which is very large in size when compared to those in the leaf blade.

The foliar veins, petioles, petiolules and young stem have a comparable type of wax pattern similar to that of the fruit wall (Plate 3.1–3.4). The folds of waxes show a thick and wavy pattern. The folds of wax are more or less parallely arranged on the vein and petiole. On these structures also no reticulum of filamentous striae are formed. The waxy coating is interrupted at the portions of stomata. In those structures the guard cells are slightly raised above the cuticle layer and hence exposed and comparatively larger in size (Plate 3).

DISCUSSION

The observations on epicuticular pattern with respect to the lower epidermis of leaf lamina, at a stage when the leaves get just hardened, agree with earlier reports (Rao, 1963; Sanier and D'Auzac, 1986). Gomez(1982) has mentioned clonal variability of cuticular ornamentation in *Hevea* and the utility of this character for clone identification. Metcalfe and Chalk (1979),

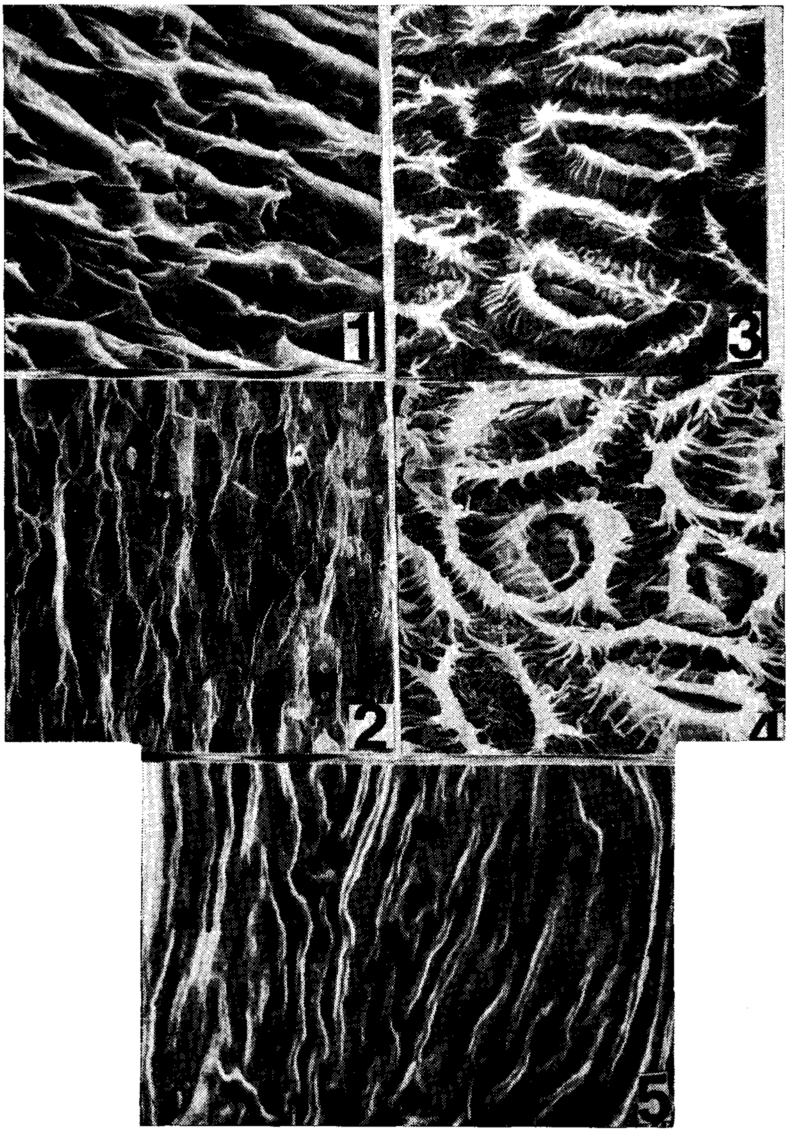

Plate 1. Developmental stages in the formation of epicuticular wax pattern on the abaxial side of *Hevea* leaf x 1000. 1. Bud break stage 2. Leaflet stage 3. Pendant stage 4. Hardened stage 5. Fully hardened stage

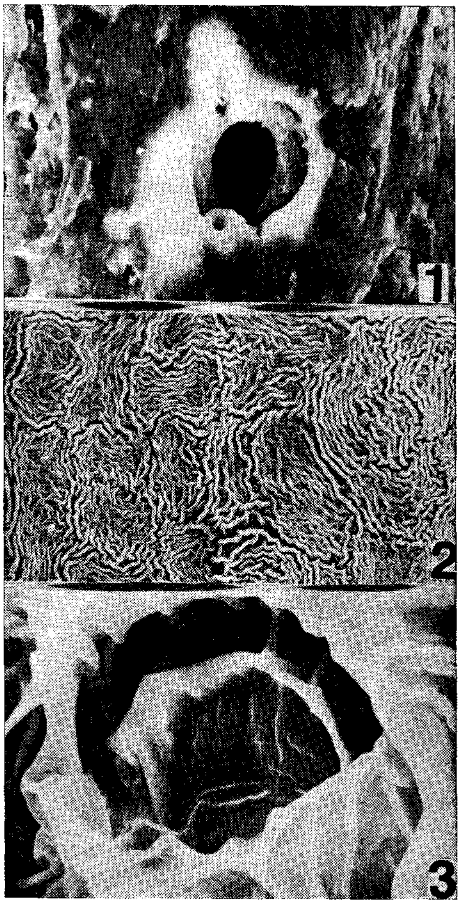

Plate 2. 1. Surface of the fruit wall of *Hevea* x 1000
2. Surface of the upper epidermis of leaf x 1000
3. A stomatal crypt x 4800

while reviewing literature on plant surfaces, have pointed out the usefulness of wax morphology as an additional diagnostic character for taxonomists. Mueller (1966) suggested that the cuticular pattern for a species is under strong genetic control and with hybridization the specific pattern breaks down and results in the appearance of recombinations.

After studying 226 species of dicotyledons, Dunn *et al* (1965) concluded that the most wrinkled cuticle is characteristic of xerophytic plants while the mesophytic or water loving species have smooth surface. Hence the striated type of epidermis in *Hevea* indicates its adaptation to xerophytic conditions. Recently, Rao *et al.* (1988) have reported clonal difference in the quantity of epicuticular wax in *Hevea* and its role in drought resistance and reflectance. The shiny appearance of the foliage of some clones can be attributed to thick and more or less even coating of soft waxes. Detailed investigation of the extent of striation and clonal characteristics may provide useful information in this regard.

As the leaves get hardened the stomatal crypts are observed sunken due to the formation of thick and prominent arms of the buttresses. Earlier reports also agree with this observation (Gomez, 1982; Sanier and D'Auzac, 1986). The present study revealed a still further development of cuticular growth in *Hevea* that further elongation and anastomosing of the striae result in the formation of very long parallely arranged tubular structures in continuous layers on the epidermis (Plate 1.5) and the already sunken stomata are almost completely covered by wax coatings. However, the thickness of wax coating is highly influenced by the environment and temperature is one major factor. In many species such as *Eucalyptus risdoni,* according to Barber (1955), the sun leaves became less glaucous than the shade leaves after a single hot day and wax formation was very high on shade leaves.

An interesting observation of this study is that the reticulum of wax forms from the pendent stage onwards, before which the wax layer is very soft and interrupted without filamentous striae. The occurrence and quantity as well as the morphology of wax are reported as important factors in the trapping of spores and hence it is of interest

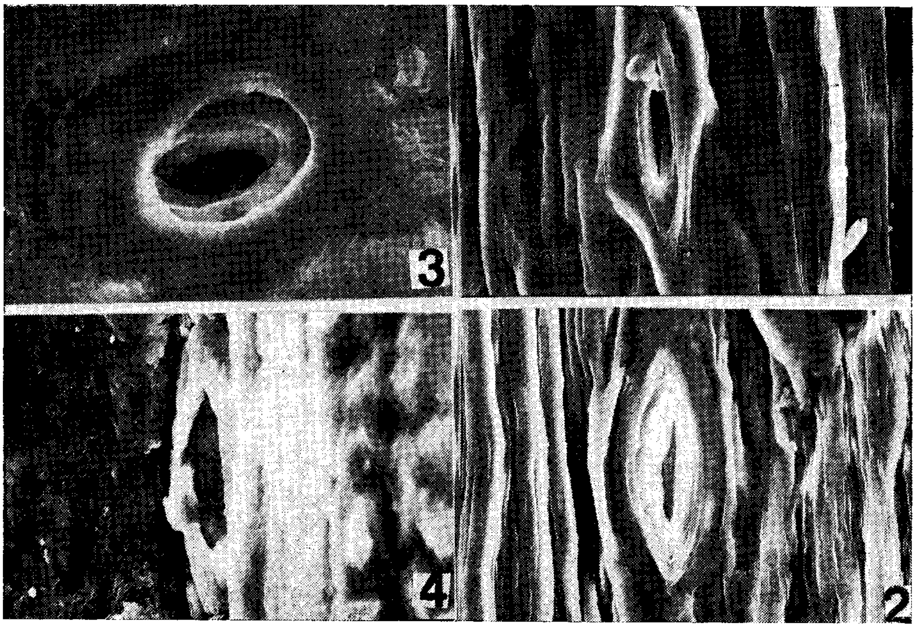

Plate 3. 1. Surface of the petiole x 1000 2. Surface of the vein x 1000
3. Surface of the petiolule x 1000 4. Surface of the young stem x 1000

to plant pathologists (Metcalfe and Chalk, 1979). It is at young stages that leaves of *Hevea* are susceptible to fungal attack, particularly by *Colletotrichum gloeosporoides* and *Oidium heveae* which are serious diseases under certain climatic conditions. Yves Senechal *et al* (1987) found that clonal difference in the resistance to *Colletotrichum gloeosporoides* in *Hevea* is significant only when the lower epidermis was inoculated. They attributed this to the complicated wax pattern on the lower epidermis. *Oidium* disease is prevalent during drought season (February-March) when the young foliage emerge during refoliation. Sufficient knowledge on the phenology of leaf and clonal characteristics can be utilised for selecting clones which skulk the infestation time and are suitable for disease prone areas. Disease management also can be made easy. The possibility of some connections between anatomical aspects and clonal sensitivity to *C. gloeosporoides* has been referred to by Sanier and D'Auzac (1986).

The organographic variability of cuticular ornamentation is clearly evident from the plates. In contrast to the sunken and covered stomata on the veinless portion of the lamina, raised and exposed stomata on the petioles, petiolules, tender stems and fruit walls are favourable sites of *Phytophthora* infection. The organographic variability of stomatal characters with respect to the leaf blade, vein, petiole and fruit wall and its bearing on part - specificity of *Phytophthora* infection causing leaf fall disease has been suggested earlier (Premakumari and Panikkar, 1984). Sanier and D'Auzac (1986) have also observed differences between leaf blade stomata and veinal stomata. The present work adds conformatory evidences in support of the relationship between the epidermal structure and part-specificity of *Phytophthora* infection on the basis of the architecture of epi-cuticular wax.

The study provides information on the general pattern of epicuticular wax in *Hevea* on the upper and lower epidermis. The striated type of wax growth indicates adaptive nature of this species to drought conditions. Along with the quantitative aspects, clonal variations in the pattern and extent of striations, if investigated, may be of use for clone identification and selection of clones for stress conditions. Information on the developmental stages of wax formation in relation to the phenology of leaves can be very well utilized in disease management and clone selection. However, the organographic variability in the epi-cuticular wax pattern and topography of stomata provide clear explanations for the organographic specificity of *Phytophthora* leaf fall disease in *Hevea* in the light of earlier work.

ACKNOWLEDGEMENT

The authors are very happy to express their sincere thanks to Shri. Peter Kosy, Head of the Instrumentation Unit, Regional Research Laboratory of the CSIR at Trivandrum for his sincere help and co-operation for the scanning work.

REFERENCES

Anonymous (1976). Branch induction of young trees. *Planters' Bulletin*, **147**: 149–158

Barber, H. N. (1955). Adaptive gene substitutions in Tasmanian eucalyptus. 1. Genes controlling the development of glaucousness. *Evolution*, **9**: 1–14.

Chambers, T. C. & Possingham, J.V. (1963). Studies of the fine structure of the wax layer of Sultana grapes. *Australian Journal of Biological Sciences*, **16** : 818–825.

Clark, J. A. & Levitt, J. (1956). The basis of drought resistance in the soyabean plant. *Physiologia Plantarum*, **9**: 598–606.

Dunn, D. B., Sharma, G. K. & Campbell, C. C. (1965). Stomatal patterns of dicotyledons and monocotyledons. *American Midland Naturalist*, **74** : 185–195.

Gomez, J. B. (1982). Anatomy of *Hevea* and its influence on latex production. Malaysian Rubber Research and Development Board, Kuala Lumpur. p. 58.

Gururaja Rao, G., Devakumar, A.S., Rajagopal,R., Annamma, Y., Vijayakumar, K.R. & 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(2)**: 84–97.

Metcalfe, C. R. & Chalk, L. (1979). Anatomy of the Dicotyledons. 2nd ed. Clarendon Press, Oxford, pp. 276.

Mueller, S. (1966). The taxonomic significance of cuticular patterns within the genus *Vaccinium* (Ericaceae). *American Journal of Botany*, **53**: 633.

Premakumari, D. & Panikkar, A. O. N. (1984). Organographic variability of stomatal characters in *Hevea brasiliensis* Muell. Arg. and its possible significance in clonal susceptibility to leaf fall disease. *Proceedings of the Internationl Rubber Conference, Sri Lanka*, 1984. pp. 317–324.

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

Sanier, C. & D'Auzac, J. (1986). Anatomical study of the epidermis of the *Hevea brasiliensis* kunth. (Muell. Arg.) leaf. Comptes Rendus de 1 Academic des Sciences III (Sciencies de la via). 303 (8) 325–330. Universite des Sciences et Technique du Languedoe. Montpellier cedex 34060, France.

Yves Senechal, Cristine Sanier, Eric Gohet, & D'Auzac, Jean (1987). Different modes de Penetration due *Colletotrichum gloesporoides* dans les feuilles d' *Hevea brasiliensis*. Physio-logic vegetable/Plant Physiology. C.R. Academy of Sciences, Paris. 5 305, Serie III, pp. 537–542.