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PLANT PHYSIOLOGY - Anatomical study of Hevea brasilliensis leaf epidermis

The lower epidermis of leaves from Hevea, already before complete maturity, presents a rough architecture and a very rugged structure which confers it a strongly reticulated aspect. Most of the stomata found in the lower epidermis are deeply seated in that reticulate structure while other stomata-like structures, less in number, and situated on the veins are completely exposed. The upper epidermis, devoid of stomata, presents at early stages a very fine architecture conferring it a striated aspect, the whole developing into a finely entangled vermicular network at the adult age.

In the ligneous of decaying leaves, the young leaves are generally more sensitive to fungal attack than the older leaves. This is particularly the case for the Hevea regarding a pathogenous mushroom, the Colletotrichum gloeosporioides (Penz.) which leads to notable damage in the eco-climatic conditions of certain plantations in Camaroons.

The object of this study is to understand the ontogenesis of foliar development of the Hevea at epidermic plane, so as to attempt to connect the anatomic evolution to the sensitivity to pathogenes fungus and to find out the connections, if any, between the anatomic aspects and the clonal sensitivity incidentally known, from other sources at least for the C. gloeosporioides (Y.Senechal and A.Beltoise, private correspondence).

Different techniques have been used to bring out the lower epidermis and its stomata:

- the first one consists of making the finest possible cuts by hand, of the lambeous of epidermis, with or without Lugol colouring and mounting in water between blade and slide;

- the second technique is by treating with a solution ion of 3% of KOH in boiling water, in which we dip the fragments of the leaf. After few minutes the specimens are rinsed several times with tap water and then we can easily remove the upper or lower epidermis on the aged leaves which we observe as in the earlier case.

These techniques have enabled to observe the longitudinal paradermal cuts in photonic microscope. Finally, to confirm the first observations, the small fragments of leaves have been fixed with F.A.A. then dehydrated with alcohol by successive baths, treated next with critical point method and metallized with the gold-palladium before being observed through a scanning electron microscope "JEOL JSM 35".

The vegitable based material coming from a tropicalized of the I.R.CA.-C.I.R.A.D. at Montpellier. Though the stomatic density differences were noticed (2), a superficial study of the adult leaves of few clones available at site have not showed the evident qualitative clonal difference, in first approximation, which led us to use the clone GTI essentially in this work.

The leaves called "aged" have been taken at stage D(5) (green leaf, habit raised) length of more than 10 cms; the "young" leaves at B2 stages (small red hanging leaves) and C (clear green hanging leaves) of less than 5 cms length.

Photos on Sheets I and II are as explained below:

(refer original)

The epidermis of the Hevea brasiliensis leaves has been subject of limited number of anatomic studies (1) to (4) using the photone microscope alone. The first

observations of Rao (1) which showed a cuticle of reticulated type on the surface of the lower epidermis of the leaf the only one to possess the stomatas, have been confirmed here (Fig.1, 2, 3, 4).

The examination with the optical microscope, of the lower epidermis has enabled to bring out that the reticulated cuticle, very much, emphasized at the surface of the adult leaves, which is unnoticeable in young leaves, shows itself in the form of crests, which are more or less ramified (Fig.3, 5). Each crest seems to surmount an epidermic cell and seems to be oriented parallely to the biggest length of this cell. If we make the focus of the microscope to vary, and when we penetrate the cellular layers, the crests disappear and the epidermic cells with sinuous refringent contours are clearly visible, as well as the stomates situated in the same plane (Fig.4, 6). These latters are bordered by two subsidiary cells and thus are of paracytic type (1, 6). The examination of samples with scanning electronic microscope confirms the existence of differences at cuticle level between young leaves and aged leaves (fig. 7, 8). The images relief clearly show the crests of the cuticle, which protect the stomatas of aged leaf by encrypting deeply seating (Fig.9, 10). At B2 state of young (Fig.11) the epidermae and stomatic cells are indiscernable; they become partial next, in state B2 of aged (Fig.12) and perfect, at the end of B2 state (Fig.13).

On a leaf which is a little less yellow, at the end of B2 state, but still anthocyanic, we still notice the contour of the epidermic cells, but already the cuticle begins to be reticuled (Fig.7, 13). The stomates are clearly visible at this state (Fig.14).

At state C, an young green leaf (Fig.9) shows the same cuticle as an aged leaf (Fig.10). It is to be noted that

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the structures comparable to the stomates and located on the veins, of precocious appearance, are not at all protected by the epidermic crests. At B2 state of young (Fig.11), the epidermic cells which surround these stomates seems pushed back; the two subsidiary cells are indiscernable (Fig.17). The "vein stomates" are also found on aged leaves with the cuticular crests clearing the stomate instead of encrypting it (Fig.17). In first approximation, they represented less than 3% of whole of the stomes. At states C or D, though the stomates be very encrypted and thus protected under the cuticular crests (Fig.16), occasionally we can notice the pores of stomates in the light of cavities (Fig.15). It is then possible to distinguish an edge in bulging on the stomate, forming a first rampart protector and finally a second rampart constituted by the cuticle of guard cell (Fig.15, 16).

A quick study with scanning microscope has also been carried out on the upper epidermis of the Hevea leaves. This shows the presence of a pleated cuticle whose evolution we can follow. On a very young leaf, at the beginning of state B (Fig.18), we can hardly distinguish the contour of the epidermic cells and a very light pleating at their surface. At B2 state (Fig.19) the contours are sharply visible, the cells are bulged; with numerous folds decorating their surface. These latters seem more or less oriented in the same direction and parallel, for a same cell. In an adult leaf (Fig.20 we still arrive to follow the contours of the epidermic cells but the pleating of the cuticle at their surface, is much more confused. The folds seem tangled. The transversal sections (figures not given) show that the thickness of the cuticle on the lower epidermis varies approximately from simple to double from the yellow leaf to that of the aged (from 5 to 10 µ), following the formation of crests previously studied. On the upper epidermis, we do not notice slight variation of thickness of the cuticule; the modifications following the maturing of the leaf seems reduced to an exacerbation of

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pleating of the cuticle at the surface and not of the thickness (from 2 to 3 µm also for an yellow leaf as well as for an aged leaf). Finally, the observation of the edge of the leaf, shows for the lower epidermis, much higher cuticular crests, less closer from one against the other; on the other hand, the cuticle of the upper epidermis is formed by a network of folds, dense and compact but less thicker.

CONCLUSION

This preliminary study confirms that the lower epidermis of the leaf of the Hevea brasiliensis is the only one to possess the stomates. At adult state, this epidermis possesses a system of thicker crests; reticulated, these can constitute a bigger mechanical obstacle to the penetration of pathogenous fungus. This system is non-existent at the juvenile state and becomes very much pronounced only at state C. The deep seating of stomates, present on the only lower face of the leaf, could eventually constitute an obstacle to the penetration of pathogenes. However, the existence of "vein-stomata", whose access is much more easier, could be (on the other hand), a preferential way of access.

The cuticle of upper epidermis shows, at yellow state, a strongly serrated aspect, leaving the epidermic cells clearly separated. At adult state it appears in the form of a vermiculated network finely tangled no more allowing to perceive the contour of the epidermic cells.

It now seems important to find out whether the differences of clonal sensitivity to C. Gloeosporioides can be connected to the differences of anatomic structure. In the contrary case, the clonal chemical factors, such as the variations in the contents of phenolic compounds of leaves (for example) could be taken into account.

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