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Development of Epidermis on Banana Fruits

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Abstract. Fruit epidermis of *Musa* (AAB) cv. Poovan (S) remains single-layered throughout its development. There is no change in stomatal number but its frequency and index decrease due to slight increase in epidermal cell number and size. The external wall of the epidermal cells shows stratification of wall layers that is characteristic of normal epidermal cell with cuticle and epicuticular wax deposits. Surface wax deposits show qualitative and quantitative variations during fruit development and ripening.

A considerable body of information has accumulated in the past on various aspects of fruit development in banana (for recent full literature see in Stover and Simmonds 1987). However, practically no attention has been paid hitherto to the structural and histochemical changes in the epidermis. Hence this study was undertaken.

MATERIAL AND METHODS

Various developmental stages of fruits of *Musa* (AAB) cv. Poovan (S)* were collected from the same population of plants growing under identical climatic and edaphic conditions. Twenty fruits in each developmental stage were sectioned at a thickness of 8–25 μm in a cryotome immediately after processing them through customary methods of fixation, dehydration and embedding. In addition, thin epidermal peels were taken with a sharp razor blade. Both the sections and the peels were stained with the following dyes; Alcianblue 8GX (Quintarelli *et al.* 1964), Auramine O (Gahan 1984), Krajcinovic amine reaction

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* All the edible banana cultivars are triploid and originate from one of the two wild diploid cultivars, *M. acuminata* and *M. balbisiana* or the combination of their two genomes. It is, therefore, customary to indicate the cultivar by the generic name *Musa* followed in parenthesis by the genome constitution. A refers to the genome contributed by *M. acuminata* and B refers to that contributed by *M. balbisiana* (Simmonds and Shepherd 1955).

(Pearse 1968), Periodic Acid-Schiffs' reagent (PAS) (McCully 1966), Ruthenium Red (Johansen 1940), Sudan IV (Comori 1952), Tannic acid-Ferric chloride (Foster 1934) and Toluidine blue O (O'Brien *et al.* 1964). Materials stained with Auramine O were examined in a Nikon epifluorescence microscope with a blue filter.

Imprints of the surface, avoiding the extreme base and the tip of the fruit, of the developing fruits of various stages were prepared using cellulose acetate, colourless nail polish, thermacol (packing material) dissolved in xylene, silicon rubber or glycerine) gelatin (O'Brien and McCully 1981) and frequency of stomata, stomatal index and size of the epidermal cells were calculated.

Small blocks of fresh materials, after critical point drying, were mounted with fruit surface on top on specimen stubs using electrically conductive paste and then coated with gold for a thickness of about 0,2 μm in gold-coating instrument. These were scanned using a Hitachi-S415A Scanning Electron Microscope at 15 kV operation current and 60 emission current (Dayanandan and Kaufman 1973). The quantitative and qualitative changes in the surface waxes of the developing fruits were studied according to the procedures given in Holloway and Challen (1966). The wax content of 16 d and 48 d old fruits was analysed by gas chromatography using OV-1 column with N_2 as carrier gas with a flow rate 40 ml min^{-1} FID raising the temperature of the column from 70 °C to 300 °C at 6 °C min^{-1} using GC Hewlett-Packard 5890.

RESULTS AND DISCUSSION

The epidermis remains single-layered throughout fruit development and possesses only stomata and unspecialised epidermal cells. The latter are squarish to polygonal in surface view.

Stomata

The stomata are of Rhoeo type (Figs. 1, 4, 7), as already mentioned by Roth (1977), with four subsidiary cells, two lateral and two or occasionally three polar cells. The stomata lie parallel to the long axis of the fruit and fairly sunken (Fig. 2). The same type was also recorded for the banana leaf (Tomlinson 1974). Cuticular ledges are present on the guard cells both on the inner and outer walls (Fig. 2). In a detailed survey, Rajagopal and Ramayya (1979) classified the stomatal ledges of *Musa* to Group I type 4 of their classification where cuticular ledges were present on either end of the guard cells as seen in transectional view of leaf. The stomata of banana fruit studied here had similar ledges. The guard cells are with chloroplasts and with starch grains which lie in two curved rows. The guard cells, as well as the entire stomatal apparatus, increase in size with age both in longitudinal and transverse directions.

Table 1
Epidermal cell frequency, cell size and stomatal frequency

	Fruit age [d]						
	8	16	32	48	72	96	120
Epidermal cell frequency [mm^2]	6093	5945	5545	3465	2985	2125	1810
Epidermal cell size (surface view) [µm]							
Perpendicular to the long axis	11.5 ± 1.8	14.4 ± 1.3	24.5 ± 2.3	27.5 ± 3.1	27.7 ± 3.4	28.7 ± 3.4	39.7 ± 3.9
Parallel to long axis	10.4 ± 2.1	13.2 ± 1.6	14.1 ± 1.5	14.7 ± 2.2	16.6 ± 3.0	17.8 ± 2.5	20.1 ± 2.5
Epidermal cell size (Transectional view) [µm]							
Tangential	11.5 ± 1.2	15.0 ± 1.4	22.6 ± 3.1	23.6 ± 2.9	24.0 ± 5.6	26.1 ± 3.2	38.4 ± 2.3
Radial	18.0 ± 1.4	16.5 ± 1.3	24.0 ± 2.8	23.4 ± 3.2	20.4 ± 2.3	21.3 ± 2.3	22.2 ± 1.3
Stomatal frequency [mm^2]	65.0 ± 7.5	45.6 ± 4.6	30.3 ± 3.5	10.8 ± 1.3	8.0 ± 1.2	7.0 ± 1.2	6.0 ± 1.2
Stomatal index	17.3×10^{-3}	14.7×10^{-3}	12.8×10^{-3}	6.7×10^{-3}	5.1×10^{-3}	4.1×10^{-3}	3.3×10^{-3}
Stomata [µm] length	30.0 ± 4.7	34.5 ± 1.5	39.5 ± 5.3	46.5 ± 1.4	46.8 ± 5.6	48.6 ± 4.6	53.6 ± 4.8
[µm] width	30.8 ± 2.1	30.0 ± 2.1	48.8 ± 7.3	52.7 ± 6.3	56.0 ± 2.8	65.6 ± 6.4	75.6 ± 12.3

Reduction in stomatal frequency per mm^2 observed during fruit development is due to this increase in stomatal and epidermal cell size (Table 1). No new stomata are differentiated during fruit ontogeny, the stomatal index gets reduced due to increase in the number of epidermal cells by cell division. In surface view, many cuticular denticulations are found to extend laterally from the guard cells towards the lateral subsidiary cells (Fig. 4) and these are found to be in line with the very thin waxy threads that extend throughout the width of the lateral subsidiary cells (Figs. 4, 7). The wall facing the stomatal opening of the guard cells is more fluorescent than the outer one and stains blue with Toluidine blue O. The stomata in the young fruits remain either fully open or variously closed while in old fruits all stomata are fully open.

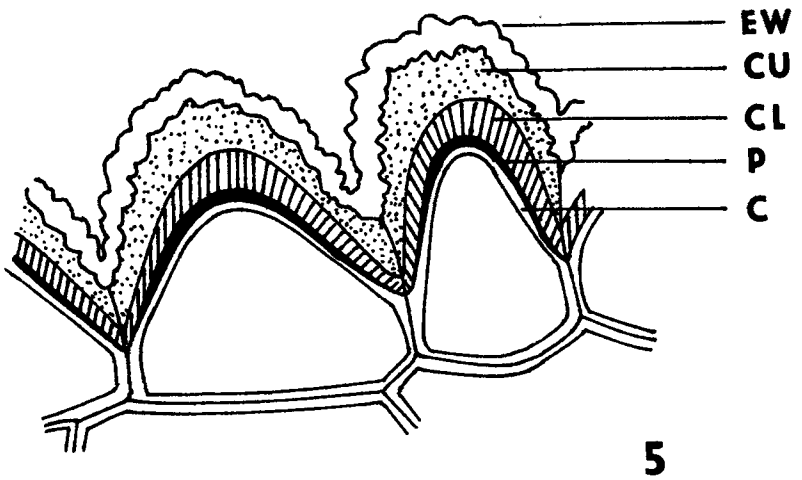

Fig. 5. Diagram of the different layers of the outer tangential wall of epidermal cells.
C – Cellulose, CL – Cuticle, CU – Cutinized layer, EW – Epicuticular wax, P – Pectin.

Epidermal cells

The epidermal cells are squarish to polygonal in surface view (Fig. 1) and conical, dome-shaped, hemispherical or bottle shaped in T. S. (Figs. 2, 3) and do not have sinuous walls. The cells are usually uninucleate with a lining layer of cytoplasm and are devoid of chloroplasts; occasionally some cells are binucleate. Increase in number of cells occurs initially up to 6 weeks and gradually cell division rate reduces and finally comes to a stop at about 13 weeks. Cells increase in number predominantly along the long axis of the fruit. Size of the epidermal cells increase appreciably with the age of the fruit as evident from the frequency data. Cell expansion occurs mainly in a tangential direction especially in the ripe fruit and hence the cells appear crushed.

In transections, the epidermal cell walls reveal five layers on the outer face of the cells (Figs. 2, 3, 5). The outermost is a fairly thick, pale and amorphous

epicuticular waxy layer (EW) that stains predominantly with Auramine O. The second layer (CU) is cuticularised and contains, in addition to cutin (positive reaction to Auramine O, Sudan IV and PAS), some pectins (as indicated by positive reactions to Toluidine blue O, Alcian blue, Tannic acid-Ferric chloride, Ruthenium Red and amine test). The third layer (CL) is the most deeply staining cutinised layer and contains a major amount of cutin, cellulose and some pectins. The fourth layer (P) is positive to all pectic stains and is intensely pectic; it does not contain cutin but cellulose is found to some extent. It forms a transition between the cuticular membrane and cell wall proper. It is the pectic middle lamella of the outer periclinal walls of the same cell. The fifth and innermost wall layer (C) is very thin and primarily cellulosic, although some pectins could be demonstrated histochemically. Our results are in agreement with the schematic structures of the general cuticular membrane provided by Martin and Juniper (1970).

The epicuticular wax

In the ordinary epidermal cells the epicuticular waxes show a reticulate pattern of deposition (Figs. 1, 4); the deposition is more along the periphery of the cells than towards the centre of the cell. In a number of cells of the developing fruit, just before the initiation of ripening, the central thin waxy region becomes depleted of wax and in that place a more or less circular pore is formed (Figs. 6, 7). In this region, the layers (CU) and (CL) mentioned above also get dissolved exposing the pectic layer (P). Pore formation has no relation to the size of the cells and could be seen in epidermal cells of diverse sizes. The pore is lined by a definite rim of thickening material (Fig. 7) which is only Sudan positive indicating the presence of cutin alone. The fact that irrespective of their previous nature, all stomata become completely opened almost along with this pore formation, indicates that the two phenomena together are related to the initiation of active climacteric respiration in the developing fruit. The two together probably facilitate greater gas exchange.

The guard cells have a thin and smooth waxy deposit (not reticulate as in epidermal cells). The subsidiary cells are interesting in the sense that the lateral ones have very thin waxy threads that extend perpendicular to the long axis of the guard cells; mild reticulation of wax deposition could be noticed on these subsidiary cells on the side away from the guard cells (Fig. 1). The terminal or polar subsidiary cells resemble the other epidermal cells in the type and amount of wax deposits (Fig. 1). It is likely that polar subsidiary cells originate from ordinary epidermal cells because of the same wax pattern which they show. The guard mother cell and the lateral subsidiary cells might be the result of a different origin from a common meristemoid because of almost identical wax pattern in them.

Quantity of epicuticular wax increases twofold when the fruit becomes exposed out of the bract, *i.e.*, at 8 days. By about the 16th day, however, wax content gets reduced slightly. This amount is maintained almost upto maturity but during the initiation of ripening wax quantity further gets reduced by about half the amount. Thin layer chromatographic studies indicate the presence of alkane, n-aldehyde and n-primary alcohols in the wax of the young fruits and alkanes, β diketones, n-secondary alcohols and hydroxy β diketone in the mature fruits, just before ripening. The qualitative changes in the epicuticular wax observed during development and ripening are probably related to the specific odour emanating at the time of ripening. Luckner (1984) reported that the characteristic smell of banana fruit is due to amyl-acetate and amyl-propionate production from the waxes. Probably in this cultivar also the qualitative changes in the wax content detected by gas chromatography are related to ripening and odour production.

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Figs 1, 2, 3, 4, 6, 7 at the end of the issue

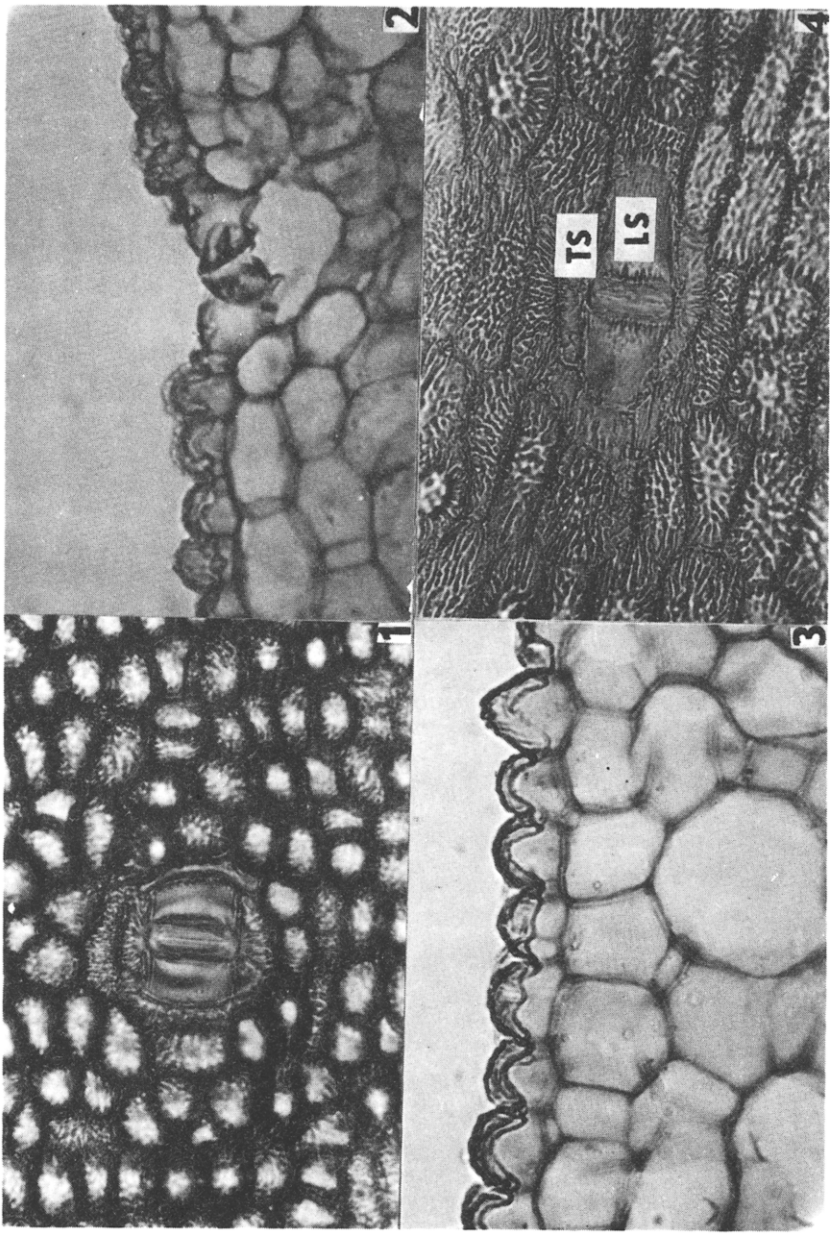
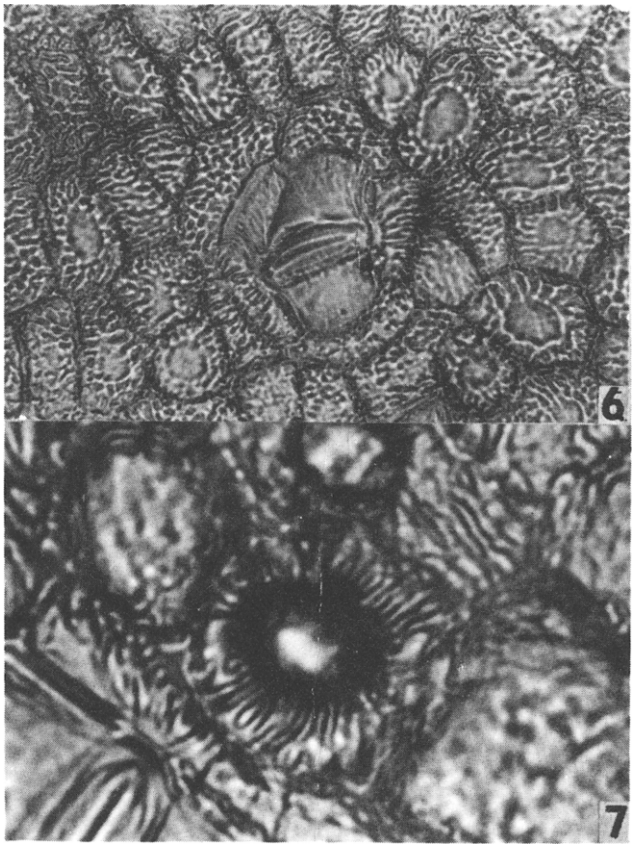

2
4
TS
LS
1
3

Fig. 1. Cellulose acetate imprint of a young fruit epidermis showing *Rhoeo* type of stoma. The epidermal cells are squarish to polygonal in surface view. Note the presence of reticulate wax deposits on the epidermal cells and on the polar subsidiary cells.

Fig. 2. Transverse section of young fruit showing sunken nature of stoma. The different layers of the outer wall of epidermis as described in text are visible. The guard cells show cuticular ledges both on the inner and outer walls. Note the different shapes of the epidermal cells in transectional view.

Fig. 3. Transverse section of young fruit showing differently shaped epidermal cells. Here also the different layers of the outer wall of epidermal cells as described in the text are evident.

Fig. 4. Cellulose acetate imprint of the epidermis of a fruit older than the one from which imprint shown in Fig. 1. was taken. Note the prominent reticulate wax deposits in the epidermal cells as well as the difference in wax deposition between terminal (TS) and lateral (LS) subsidiary cells. All figures 400 ×.



Figs. 6. & 7. Cellulose acetate imprints of epidermis of 48 d old fruit in lower and higher magnifications respectively showing the formation of a central pore in the waxy coating over the epidermal cell walls. The pore has a distinct rim.
6: 400×; 7: 1000×.