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November 13 - 18, 1978



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# CULTIVATION AND PRODUCTION

# ANATOMY OF TUBER AS AN AID IN YAM BIOLOGY STUDY L. Degras and P. Mathurin (\*)

# SUMMARY

In spite of the part played by the Yam tuber in the clone perennity and economical value, its anatomy remains insufficiently studied. A simple study of *D. alata*, *D. trifida*, *D. cayenensis*, *D. esculenta* and *D. transversa* sets up characters which enlighten tue species biology. In *D. alata* a very specific sclerenchymatous zone is interpreted as transfusion tissue. In all the species, cellular blocks, within the inner cortical parenchyma, make up the organizing poles of the tuber part set germination.

# INTRODUCTION AND LITTERATURE

In spite of the fundamental value of the underground tuber of Yam for its economic interest and its biology, relatively few studies concern its anatomy. Ayensu (1972) himself who mentionned this fact in his own anatomical monography considers but 18 species for their tuber out of the 67 he studied.

So, for a synthetic as well for many specific views of the Yam tuber anatomy, the best information has to call for the old thesis of Queva (1894). The normal and general types of *Dioscorea* can be thus described under four organogenetic cases (fig. 1):

A. Several Dioscorea with perennial tubers

Only cambial layers which come from secondary tissues hypertrophy; no sub-apical meristem.

B. D. alata, D. bulbifera and D. pentaphylla for instance

Cambial layers for the outer cortex and sub-apical meristem of which primary tissues relay the secondary tissues hypertrophy and make the essential tuber tissues. Delayed multiplicating layers are seen in the inner cortex.

C. D. esculenta and D. opposita for instance

A primary tissue hypertrophy is relayed by the sub-apical meristem and the cambial layer giving the outer cortex. Delayed multiplicating layers are seen in the inner cortex.

We must underline that Queva's observations rely upon germinating sexual seeds as well as on adult tuber from vegetative origin, the classification of the species which are quoted here from homology with the adult tuber of sexual origin of *Tamus* (A), *Helmia* (B), and *D. kita* (C) (see Burkill (1960) for Queva's botanic terminology.

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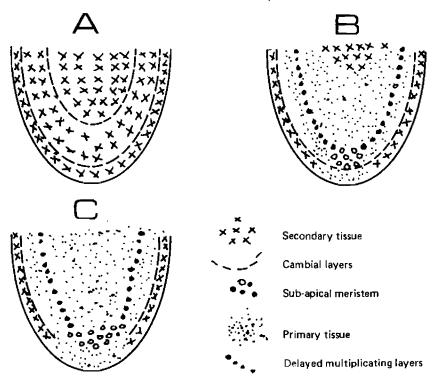


Fig. 1 - The three types of Dioscorea apex from the data of QUEVA (1894)

Apart from this general classification, we can find in Queva (1894) some details concerning *D. alata* and *D. esculenta* which will be discussed with our own observations.

Winton and Winton (1935) published a detailed account of *D. alata* structure and cell types, using the "Red Barbados" Yam.

Ayensu (1972), as we have seen did not give to tuber anatomy all its importance and this is again reflected in the fact that the gives a unic description for the whole *Enantiophyllum* section which contains *D. alata, D. cayenensis* (x), *D. opposita* (= *D. batatas*) and *D. minutiflora*, this last one being a tuber perennial species. So its description is vey general. As well, we might use it as the general frame of Yam tuber anatomy. The more so that the section *Macrogynodium*, which hold *D. trifida*, does not bring great variations. From the periphery to the center, the following structures are seen:

- a primary tissue outer cortex of irregular suberified cells
- a secondary tissue outer cortex of numerous suberified layers in more or less radial rows
- a fundamental inner cortex tissue of parenchymatous cells sometimes in radial or stratified series
- a fundamental central amyliferous parenchyma where collateral (stem type) vascular bundles are dispersed.

Raphids and tannin cells are seen.

(x) We use the Flora of West Tropical Africa (Miege, in Hutchinson, Dalziel, Hepper, 1968) terminology, where we have *D. cayenensis sp.* comprising ssp. cayenensis and ssp. rotundata.

Occasionnal anatomical tuber data has been met in litterature, the most interesting and recent of which are those of Onwueme (1973) and Mantell, et al. (1977). Sharma (1974) alone, published a detailed contemporary study of tuber anatomy, but of a wild *Enantiophyllum* species, *D. Glabra*.

As could be foreseen from a so limited interest in tuber anatomical study, many observations remain superficial and lead to many discrepancies either by lack of specification of given or by speculative description of the same structure. The most exemplary case is the *D. alata* one, in spite of the outstanding position and dispersion of the Great Yam in the whole tropical area.

Ayensu (1972) speaks of the "striking uniformity of the general anatomy". So he never mentionned the lignified structure of the inner cortex which for Mantell et al. (1977) is quite distinctive of *D. alata* one from that of *D. cayenensis*, in spite of their *Enanthiophyllum* grouping. Queva (1894) holds for oblical suberified layers in the *D. alata* cortex and for indifferentiated bunldles among an inner cortex layer of thick wall cells and Winton and Winton (1935) for a pericycle of stones cells filled with an oxalate crystal. None of these authors localised the "meristematic layer" of Onwueme (1973) from which neoformed buds are rising in the common seed pieces in which most cultivated yams are cut for plantation.

The general investigation we conduct of the Yam tuber fragmentation effects (Mathurin, Degras, 1974, Degras, Mathurin, 1975) has to take account of the anatomical structures and evolution which permit the bud germination and growth. And, as can be seen from the present review, no sound data could be obtained from the litterature. Hence the following observations.

# MATERIAL AND METHODS

# Cultivars

We first observed the cultivars used in the quoted investigation; *D. alata* cv "Pacala" and "*D. trifida* pv "INRA 25", then in front of the surprising differences, we extended our observations to *D. cayenensis*, *D. esculenta* and *D. transversa* cultivars. Here are some traits of them.

D. alata cv "Pacala" is known from Guadeloupe. Its African secondary origin may be related to the Pakalla community of Ivory Coast. Similar clones are known in Dominica. The tuber is more often cylindro fusiform, rarely finger-tipped, white-fleshed, with a rather midly cracked corky bark. It has a relatively long storage ability (Degras et al. 1972).

D. trifida cv "INRA 25" is an hybrid clone selected from a cross done in Guadeloupe in 1966 and released in 1971 (Degras et al. 1971). The pyriform tubers may reach as much as a fifty per plant and a mean weight of 100-250g per tuber. The flesh is white and of high cooking grade. The skin is rather thin with thickened and cracked transversal corkly lines. The storage ability is very short (Degras et al. 1971, Martin and Degras, 1978a).

- D. cayenensis ssp. rotundata cv "V17-2" has been introduced from West Africa (may be Cameroon) about the year 1964 by IRAT, (x) and pass to INRA collection in 1966. The clone as a flesh creamy whitish colour which should place it not far from a ssp. cayenensis. It is of early first harvest and, as usual, this first tuber is not stored very long. It was our material.
- D. esculenta cv "Pas-possible" is a "Chinese yam" introduction of the end of the last century from Indo-China peninsula. Like "INRA 25" the underground tubers are clustered but their peduncle is thin and the tuberized part is cylindroid with a smoother and thinner corky bark. They can be stored for a longer time than "INRA 25" but less than "Pacala".
- D. transversa cv "Waë!" has been introduced in 1969 from New Caledonia under the false name of D. nummularia. This minor species, at the world level, is highly appreciated in its country (Bouret, 1973) and appears susceptible of a wider dispersal (Martin, Degras, 1978b). Its tubers are several per plant, with a long neck, and covered with rootlets; the skin is thin and cream coloured, the flesh white at the center under a slight pinky-purple peripheric zone. It is of fair cooking behaviour and good taste. It keeps easily in the soil and seems able of a long growth while it is of a long growth while it is of very short storage aptitude, mainly through bruising accidents.

# Methods

In this preliminary approach, only adult and normal but rather small tubers has been observed. It follows different tuber weight, respectively 150 to 400g, for *D. trifida*, *D. esculenta* and *D. transversa* but 500 to 800g for *D. alata* and *D. cayenensis*.

Transversal middle section has mostly been used with a limited numbers of radical and tangential sections at the same tuber level.

We used the classical anatomical technique: elimination of the cell content with sodium hypochlorite, clearing in dilute acetic acid and water, colouring with alumned carmine and iodine green. Mounting was done in glycerine. Examination was conducted with an optic Leitz microscope under a 12,5 eye-piece x 3,5 objective, giving a magnification of about 150.

# **OBSERVATIONS**

They are presented in a growing complexity order and description goes from periphery to center.

Dioscorea transversa cv "Waël" (fig. 2 and 3)

(x) IRAT = Institut de Recherches Agronomiques Tropicales.

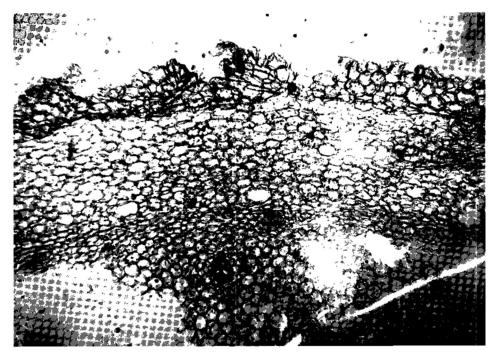


Fig. 2 – Dioscorea transversa Transversal section of the tuber

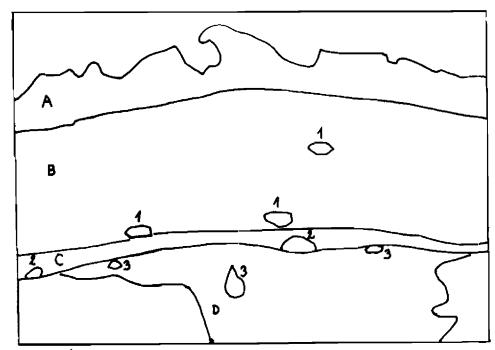


Fig. 3.— \*\*Dioscores transverss.\* Analytical drawing of fig. 1. A: cork; B: cortical parenchyma C: Inner cortex procambial zone; D: central amyliferous parenchyma; 1: raphid cell; 2: multiplicating cell blocks; 3: vascular bundle

- -5-6 layers of suberified cells, mostly irregularly disposed and isodiametric, sometimes stratified and in radial series; so, primary tissue seems prevalent in the outer cortex;
  - 1-2 poorly differentiated layers
- 10-15 layers of smaller parenchyma cells rather regularly and tangentially elongated;
   greater cells are filled with raphids;
- 5-8 layers of even smaller but less differentiated cells are also well disposed and tangentially elongated; from place to place they are interrupted by cells blocks of more or less meristematic appearance like proto phloem zones;
- ground amyliferous parenchyma with isodiametric cells growing in size towards the center and holding stem type vascular bundles evenly dispersed.

Dioscorea cayenensis cv. "V17-2" (fig. 4 and 5)

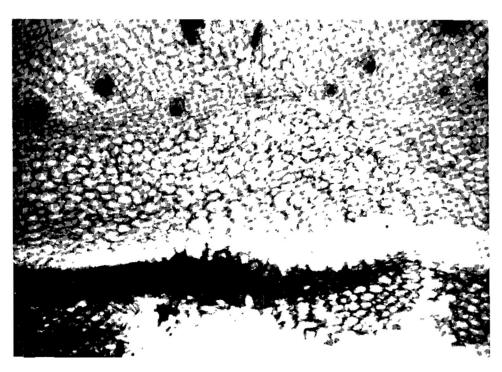


Fig. 4 - D. cayenensis. Transversal section of the commercial tuber.

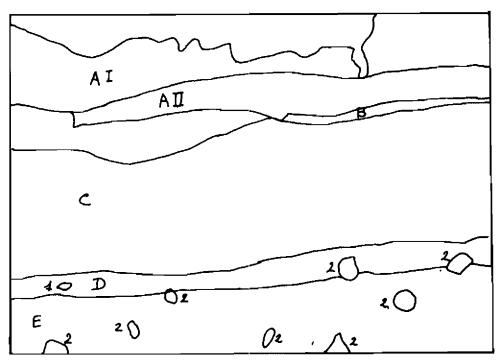


Fig. 5. - D. cayenensis. Analytical drawing of fig. 4.

Al: primary cork, All: secondary cork;

B: cambium; C: cortical parenchyma;

D: inner cortical procambial zone; E: central amyliferous parenchyma; 1: cell

blocks; 2: vascular bundle

The same succession as the precedent is observed with the following differences:

- cells are generally smaller
- the suberified outer cortex has
  - 4-5 layers of primary tissue
  - 5-6 layers of secondary cork tangentially elongated and disposed in radial series,

the inner layers being often densely compacted against a basic cambial layer

- the meristematic cell blocks seem less frequent
- associated cells in the parenchymas result in secretory chanels features.

# ${\it Miscellaneous-Cultivation\ and\ production}$

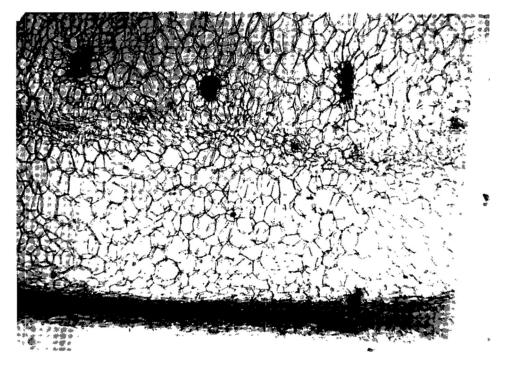


Fig. 6. D. esculenta; transversal section

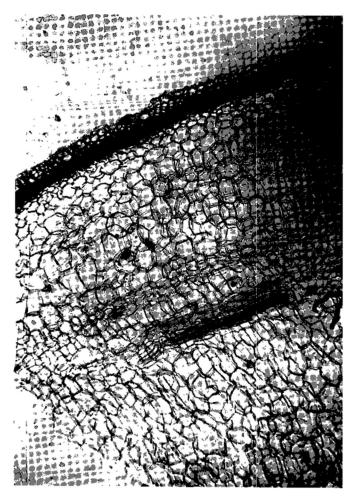


Fig. 7

D. esculenta;

longitudinal section

Dioscorea esculenta cv "Pas possible" (fig. 6 and 7)

- Suberified more or less isodiamteric cells layers partly exfolating
- Suberified tangentially elongated cell layers with thick walls resulting in unconspicious cellular content for the inner layers.
  - 1-2 cambial layers
  - cortical parenchyma with greater isodiametric cells.
  - 3-4 layers of quite undifferentiated small cells interrupted by scattered young cell blocks:
  - amyliferous parenchyma with vascular bundles.

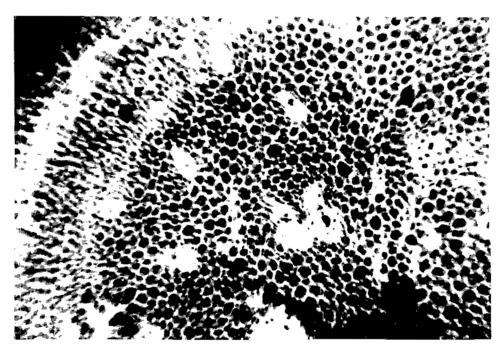


Fig. 8. - D. trifida; transversal section general view of a tuber section

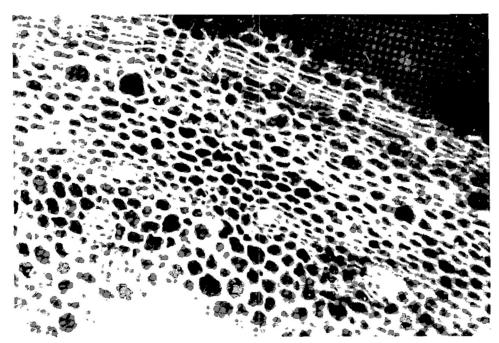


Fig. 9. — D. trifida; transversal section.

Detailed view of the peripheric zones of the tuber

Dioscorea trifida ev "INRA 25" (fig. 8 and 9)

- Suberified primary layers widely exfoliating
- 5-7 suberified secondary layers in well ordered radial series;
- 1 cambial layer linked to the suberified cells;
- 6-8 layers of cortical parenchyma with tangentially elongated cells but rarely radially associated to the precedent cambium; greater cells giving raphids or secretory systems;
  - 3-4 layers of small rather isodiametric cells, interrupted by young cells blocks;
  - amyliferous parenchyma with vascular bundles all cells growing in size towards the

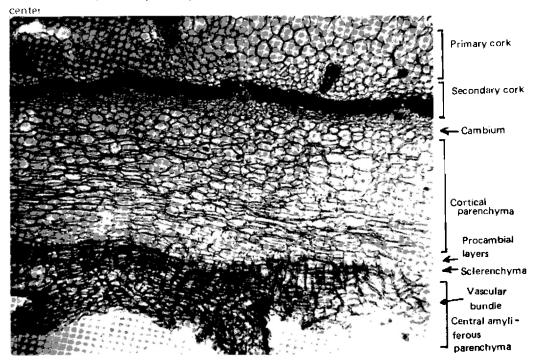


Fig. 10. D. alata. Transversal section of a tuber; general view of the peripheric zones,

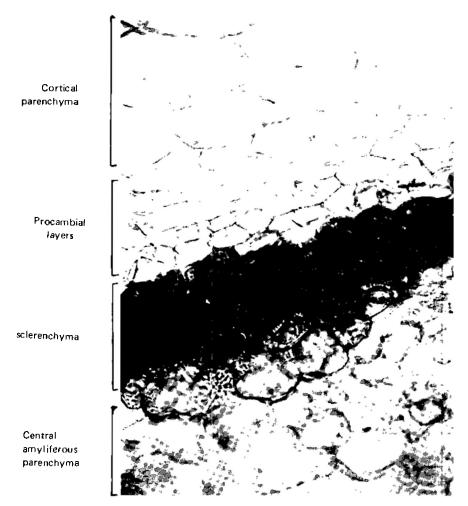


Fig. 11

D. alata transversal section; detailed view of the sclerenchyma area.

Dioscorea alata cv "Pacala" (fig. 10 and 11)

- 5.6 primary layers of large sized suberified cells
- secondary suberified layers of similarly wide cells but radially organized and squeezed on the inner side;
- 1 cambial layer associated to the secondary cork;
- 2-4 young parenchymatous layers with isodiametric cells under the cambium;
- cortical parenchyma with more or less tangentially elongated cells, rarely in radial succession; it contains secretory and raphids cells.
- 4-5 layers of small quite undifferentiated cells either isodiametric or tangentially elongated with by place young cells blocks which sometimes go in the inner tissue;
  - 3-5 layers of large polyhedric sclerenchymatous cells, giving way by place to young

small cell blocks which reach the central parenchyma; these sclerenchymatous cells are mostly with thick walls surrounding and excentered crystal, but several on the inner side in general, have relatively thin walls punctuated in the horizontal direction.

- an amyliferous parenchyma with vascular bundles of the same characteristics as the precedent ones.

# The essential traits

Over the five species we have:

- A cortex with outer corky layers
  - mid parenchymatous layers holding raphids when they exist
  - inner young small cell layers
- A ground amyliferous parenchyma holding stem-type vascular bundles.

An outer cambial layer might occur under the cork. But it is rather conspicious in some cases like *D. transversa* when the cork seemed mainly of primary nature. This leads to the variations between species.

At the cork level, besides the primary versus secondary tissue nature contribution, we notice the cell wall thickness relative variation. With consideration of the cell size it grows from *D. trifida*, *D. alata* and *D. esculenta*. The number of cell layers seems to increase from *D. transversa* to *D. trifida*, *D. esculenta* and *D. alata*.

The inner cortical layers of *D. esculenta* are thinner. And this level is achieved in *D. alata* on its singular sclerenchymatous layers.

The structural identity of the ground parenchyma through the five species is remarkable in view of the structural specific diversity of the cortex. But, it is the reverse as for the final development of each part. While the cortex, (obviously thinner towards young tuber sections) never outpass 2mm, the ground parenchyma, depending on tubers and varieties, goes from 5 to 20 cm in diameter.

# INTERPRETATION

Let us consider now the contribution which could be infered, from the anatomical structure, to the tuber functions which are accumulation, protection and re-utilization of stored biological food.

Food storage: central and cortical parenchyma

Starch grains are numerous the more so the amyliferous cells are near by the vascular bundles, which determines a macroscopic granular appearance of the ground parenchyma, the grana being the best filled cell areas. Starch grains are very few in the cortical parenchyma. Other products are mostly accumulated by this one. Raphids of calcium oxalate and tannin cells may be seen. Calcium oxalate monocrystal are filling the sclerenchyma cell lumen in *D. alata*. Are they properly food storage seems questionnable (see further).

Food storage protection: corky and sclerenchymatous layers

These layers play a protection part through different degrees of cell size and degrees of cell wall modification. And the respiratory and hydric economy of food stores as well as their mechanical protection reach different levels with each species.

The less suberified and mostly primary cork layers added to the generally thin cell walls of *D. transversa* are in accordance with the easy post-harvest decay and the relatively extending growth of its tuber. The thin cell walls of *D. trifida*, though with secondary suberified layers may be faced with the poor post-harvest conservation of its tuber, while *D. esculenta* may be more efficiently protected through the seeming high cell wall suber density of a corky cortex which is not thicker in total.

The thickness of the primary and secondary suberified layers of *D. alata* in good accordance with the rather good post-harvest conservation of its tuber. Moreover, with the sclerenchymatous layers this species may have not only a stronger mechanical protection and rigidity for deeper underground growth, but also something like the "transfusion tissue" (Boureau, 1954) which play a part in water keeping. Their cell wall punctuation and the vascular bundles vicinity are good features of that.

Food stores utilization: cortical parenchyma and indifferentiated cell block

The organogenetic area for the tuber buds is known to be at the inner cortex layer level (Onwueme, 1973, Mathurin, 1977). This area can be considered as a procambial meristem in (Boureau, 1954) sense with its sheath situation between two parenchyma (x), the frequent lengthening of its cells and its seemingly high participation in the conducting tissue.

The individual cell blocks appear as the initial multiplication site for any tuber piece outside the "head" one. This process needs energy from the food stores available. If this energy would directly come from the starch stored, the parenchyma cortex may not be adapted. Here raises the possible contribution of the calcium oxalate. At a time it was seen as only a refuse or waste product. Then data has been produced for a possible part in osmotic pression balance (Wattiez and Sternon, 1942). Others mentionned also its ability to modify the respiratory quotient (Binnet and Brunel, 1968). Now it seems to certify a detoxication process (Zinsou, 1978). Whatever may be, it apppars to be linked with a high metabolic activity. This is clear from the multiplication of raphids in the growing zones (Mathurin, 1977).

Individual cell blocks seem to function as organizing poles which structure and determine growth and morphogenesis. Their nature may be enlighted through a comparison with the undifferentiated I embryo of some seeds, and in the main part of the tuber, between harvest and germination, could exist a kind of embryonic dormancy.

<sup>(</sup>x) True procambial meristem are mostly surrounded by meaters parenchyma while we have here rather polyhedric parenchyma.

# DISCUSSION - CONCLUSION

These preliminary observations appeal for more sound research. Among needed complementary studies must be mentionned:

- comparative varietal studies within each species
- longitudinal sections
- section in tubers related to their age
- relation between cell content, mitotic activities and cell wall lignification

Nevertheless the following points can be retained as strong basis for further investigation:

- 1. The clones anatomy has specific value; this comes from the fact that our data permit a comprehensive view of those of the other authors on *D. alata*. It is now clear that this species has original hard cell inner cortical layers named by Queva: thicken cell wall layer, by Winton and Wintony: stone cells layers, by Mantell et al: sclerenchyma, and which, with its seeming transfusion tissue character may be one of the determinant wide adaptative factor of this species unic pan-topical dispersion.
- 2. The general presence of generative cell blocks offers histological basis for the structural and dynamic analysis of the tuber multiplication.
- 3. The tuber functionning (and, for essential aspects the whole plant functioning) can be understood through its anatomy, in spite of the limited transversal sections: the relative independance of the successive areas in transversal sections refers to the tuber apex (sub-apical) morphogenetical predominance, a fact which appears to emerge from perennial toward the most evolved annual types (Queva, 1894).

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