

Histological Study of the Polarity of Yam Tuber (*Dioscorea* spp.) at the Beginning of Tuberization

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Authors' contributions

This work was carried out in collaboration among all authors. Author NGA contributed to obtain the found and the subject conception. Author KCD conducted the research work and wrote the manuscript. The other two authors participated in the correction. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2019v12i230078

Editor(s):

(1) Dr. Aneta Popova, Chief Assistant Professor, Department of Catering and Tourism, University of Food Technologies, Bulgaria.

Reviewers:

(1) Folashade Ayinde, Lagos State Polytechnic, Nigeria.
(2) Nagwa Thabet Elsharawy, University of Jeddah, Saudi Arabia.
(3) Yongchun Zhu, Shenyang Normal University, China.
Complete Peer review History: <http://www.sdiarticle3.com/review-history/51369>

Original Research Article

Received 11 June 2019
Accepted 06 September 2019
Published 16 September 2019

ABSTRACT

Yam, like most roots and tubers, has a tissue variation that is responsible for the difference in cooking observed during culinary preparations. In order to understand the origin of this variation, this study was conducted through optical microscope and SEM observation of the beginning of tuberization. The local variety named Kponan of *Dioscorea cayenensis-rotundata* was used. The days after the beginning of the tuberization, the protrusion of the stem base intensified and gives rise to the tuber. The histological study of the onset of tuberization revealed once again the existence of a longitudinal growth gradient whose point of growth is the apex and the sense of maturity of the distal part at the proximal end. The cells birth at the apex and differ a little more towards the middle part of the tuber. The apex is the driving zone for the tuber's lengthwise growth. The cambial cells ensure this growth in length and thickness of the tuber. The tuber's growth activity decreases from the distal part to the proximal one. The distal part contains more vacuolated cells thus rich in water and less starch than the middle and proximal parts. The cell wall

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is thin and less lignified. The cambium generates cells at the lower end of the apex. These very vacuolated cells differentiate and will form the median and proximal parts of the tuber. The distal part will remain immature compared to the other two parts. This could explain the low dry matter and the origin of rejection or elimination of the distal part during culinary operations.

Keywords: Starch; *D. cayenensis-rotundata*; histology; yam tissue; polarity; tuberization.

1. INTRODUCTION

Yam is an annual plant whose vegetative cycle is related to the seasonal cycle. Although classified among monocotyledons, the Dioscoreacea family has a number of characteristics that can be found in dicotyledons with, for example, a cauline apical apex of the embryo (e.g., H L. Sealy 1982. Thesis, Univ. of Nantes, France). Tuber germination is associated to the first external indication of renewed growth was the development of small protuberances on tuber surface [1]. The stem of some species (*D. alata*, *D. trifida*) is winged for most of its length. This is not the case with *D. cayenensis rotundata*. The number of stems varies according to species and their size can reach 30 m of height especially in wild species [2]. The tuber, of the Latin Tuberculum, is a bulging caulinary organ, usually underground belonging to a vascular plant and at which the plant stores reserves. Sometimes it is the cortical parenchyma that becomes hypertrophic and serves as a warehouse, sometimes it is the medullary parenchyma. In fact, because of this capacity to store reserves, tubers play a nutritional role for both plants and animals [2]. Yam serves as an important source of carbohydrate (83.08% to 86.13%) and serves as a major source of income in countries where they are cultivated [3,4,5]. The world production of yam is widely dominated by West Africa with more than 92% [6]. In terms of production, green manure such as intercropping with pigeon pea improves quality and yield of tuber [7]. The technique of pre-treatment of seed by pesticides (fungicide + insecticide) improves germination rate and tuber yields [8].

The edible yam tuber has two main parts, the first of which is the pre-tuber and the second the neo-tuber [9]. The pre-tuber, called complex bud, is an unconsumable fibrous mass, from which the different organs of the new plant develop. The neo-tuber or tuber mass has three parts, the proximal zone of which is directly connected to the pre-tuber and represents the head of the tuber. The second part of the tuber (middle) is an extension of the proximal part and third, the distal part which is the growth zone of the neo-tuber [9].

Yam tuber shows a complex structure characteristic of the species. A difference on the granule size distribution [10] and yam parenchyma along the longitudinal axis [9] have been revealed. The existence of a longitudinal growth gradient observed at tuber maturity is believed to be the result of tissue variation within tuber. Cytoplasm air, cell wall thickness as well as intercellular space increased from the distal zone to the proximal part [10]. So the dry matter and starch contents are reported to be elevated in proximal and middle parts (e.g., J. Brunnschweiler 2004. Thesis, Univ. of Zurich, Suisse). This results in a firmer texture when cooked, which is better appreciated compared to the distal part. At the end of the dormancy period, the sprouting rate decreased from proximal part toward the distal portion [11]. The characterization studies on mature tuber do not reveal the origin of these variations. In the current study, we hypothesized that cellular activities could be explain these variations. So the aim of this investigation is to carried out a histological follow-up of the beginning of the tuberisation. Cell activity is more important in the initiation and growth phase of the tuber. Thus, the first month of tuberization could be allow a good perception of this phenomena.

2. MATERIALS AND METHODS

2.1 Experiment Field

The variety of *Dioscorea cayenensis*, locally named kponan (precocious variety at two harvests time) was obtained from experiment farm of Abobo-adjamé University (Abidjan, Côte d'Ivoire). Tuber pieces about 60 g were planted in nursery gardener sachet (size 20 cm x 20 cm). The tuber used as seed is constituted by the second harvest one. For this field, the head of the tuber was avoided as much as possible to allow homogeneity of the shoots.

The nursery gardener sachet has been filled up with the land of forest and arranged (0.5 m x 0.5 m). The plot of 35 m² carried out in 2013-2014, was submitted to the climate of bass cost. The mean climatic parameter is: temperature

(30.9°C), rainfall (101.5 mm), relative humidity (84.2 HW), with little monthly variation. The watering has been done when it's necessary. Three Randomized blocks design had been done.

To study the tuber polarity, the tuberization had been observed during the first month of growing. So the harvested periods were 0; 7 and 30 days after the initiation and beginning of the tuber growth.

2.2 Histological Studies

2.2.1 Light microscopy

2.2.1.1 Preparation of fixing solutions

Yam tuber was partitioned longitudinally in four pieces and each was then sectioned in three equal parts (proximal, middle and distal) (Fig. 1). The fragments were fixed in formalin-calcium of Baker. For 1L of calcium formaldehyde, 100 g of calcium chloride was dissolved in 600 ml of water. Dissolution occurs at low temperature. To this solution is added 100 ml pure formaldehyde Norma stabilized to 10% methanol. The volume of the blend is completed to 1L.

(e. g., HL. Sealy 1982. Thesis, Univ. of Nantes, France). Small cubes of yam tissue were cut into sections of 15 μ m thickness with a hand microtome of Ranvier (Holland). To improve this operation, the samples had stay for one week in ethanol-formalin acid of Locquin. This fixator has been prepared as follows; 1L ethanol, 100 ml formaldehyde, 50 ml acetic acid, 100 g sucrose and 200 ml water were blended. Yam tissue

becomes hard and easy for cutting. The thin sections were washed in water before stained.

Tuber of the kponan variety harvested 30 days after emergence in the field (appearance of the bud above the mound). The white lines indicate the direction of cutting.

2.2.1.2 Different coloring techniques

To monitor the evolution of yam tissue, the sections were coloured in ammoniacal Congo red [12]. This solution has been prepared as follows. Two % ammoniac solution (1 ml ammonia per 50 ml distilled water) has been dissolved 0.2 g Congo red. The sample is pre-treated with sulphuric acid (10 %). The cellulose thus transformed becomes acidophilic and takes on the azo colors. The cuts are then coloured for one minute in Congo red and then revealed with sulphuric acid (10%). Thus the cell membrane is stained blue and the nucleus red. A double staining was also performed. The cuts are treated with Lugol, rinsed, then stained with Congo red and revealed with sulphuric acid. This makes it possible to observe both the membrane and the cellular content.

Starch was detected by the weak Lugol reagent (2 g of pure potassium iodide per 1 g of iodine in 400 ml of distilled water) prepared according to Locquin and Langeron [12]. This reagent stains starch in blue. After the Microtome cutting step, the sample is collected in water, washed for 5 min, stained with Lugol for 3 min, and then washed in water before being observed. The mounting medium is distilled water.

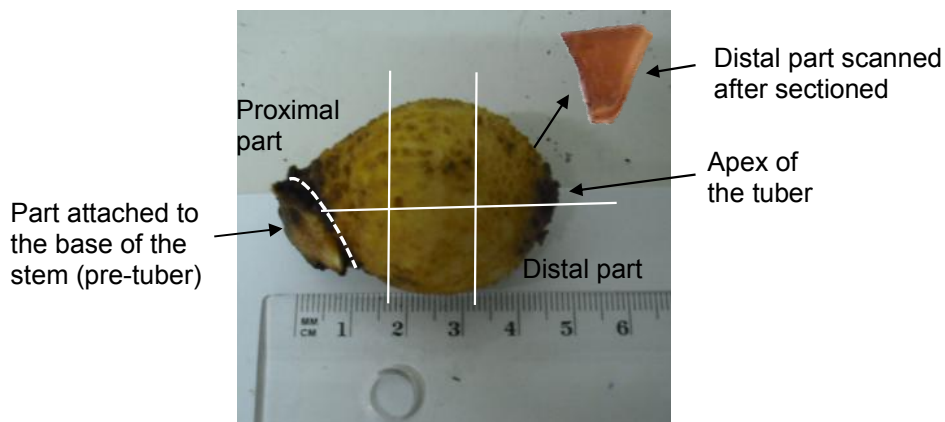


Fig. 1. Technique for cutting and sampling yam tissue for histological study

Light microscopy observations were made using an integrated camera Optika-DM 20 microscope (Italy). The resulting images were processed by Opmias Ver 1.1.

2.3 Scanning Electron Microscopy

The size of the samples fixed to formol-calcium was first reduced (0.3 mm x 0.3 mm) under a binocular magnifying lens before being dehydrated. The dehydration is carried out by successive passage in 4 ethanol baths of increasing degree (70°GL, 80°GL, 90°GL and 100°GL) followed by 2 acetone baths (e. g., HL. Sealy 1982. Thesis, Univ. of Nantes, France). The microscope Zeiss (SWPRA 40, 3148, Oberkochen, Germany) is used. As a special feature of this apparatus, the samples do not need to be covered with a thin gold film as was the case with previous models. Thus, after dehydration, the samples are mounted directly on the object plots and observed with a scanning electron microscope. The image was analysed by the software INCA drycool, Oxford.

3. RESULTS

3.1 Light Microscopy

3.1.1 Tuber initiation

The bursting stage of the suber marks the beginning of tuberization. The cross-section (Figs. 2A, B) shows the anatomical structure of

the visual initiation of tuberization. The apical end of the stem initiates an outgrowth that spreads around the stem. From the outside to the inside, three areas stand out. The first part stained blue by lugol reagent consists of the meristem cells whose division activity results of the growth of the neo-tuber. The second area coloured yellow by the lugol consists of a draft of conductive tissue oriented in the direction of tuber growth and the third one corresponds to the fibrous inner part of the pre-tuber. It is difficult to identify the cell wall of the meristematic cells as well as the conductive elements, because the cut is thick. Calcium oxalate crystals in an orange-coloured are observed on the entire surface of the section.

3.1.2 Seven days after tuber initiation

The longitudinal section of the neo-tuber (seven day-old) stained by lugol reagent showed the basal stem, the root, the tuber initiation and the marrow of the stem colored in blue dark (Fig. 3A). The tuber nascent is situated on the basal extremity of the stem. The sample of Fig. 3B was longitudinally and transversely sectioned to study yam tissue formation.

The days following the tuber initiation, the basal extremity of the stem continues its growth in radial and longitudinal directions. The transversal section of the neo-tuber (Fig. 3C) showed the meristem activity and the first undifferentiated tissue such as conducting vessels (Fig. 3C, D, F).

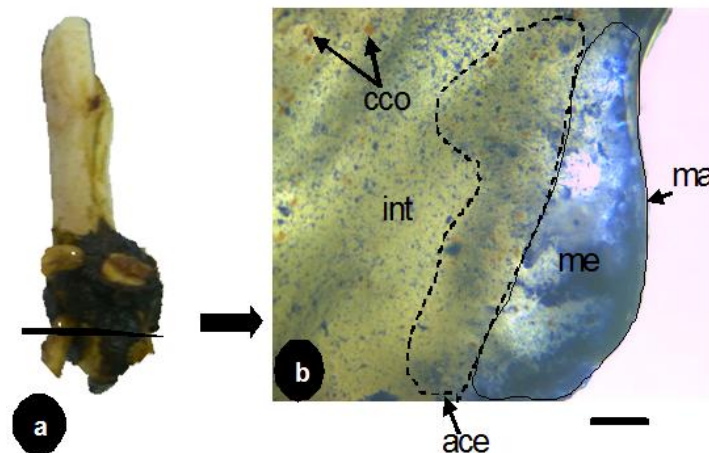


Fig. 2. Cross-section of the bursting stage of the suber of the yam tuber of the kponan variety. The draft tuber with its stem (a) shows in this section the beginning of the tuberization (b). cco, crystals of calcium oxalate; int, inner of the tuber; ace, area of conductive elements; me, meristem; ma, meristem area. Scale bars: 25 µm

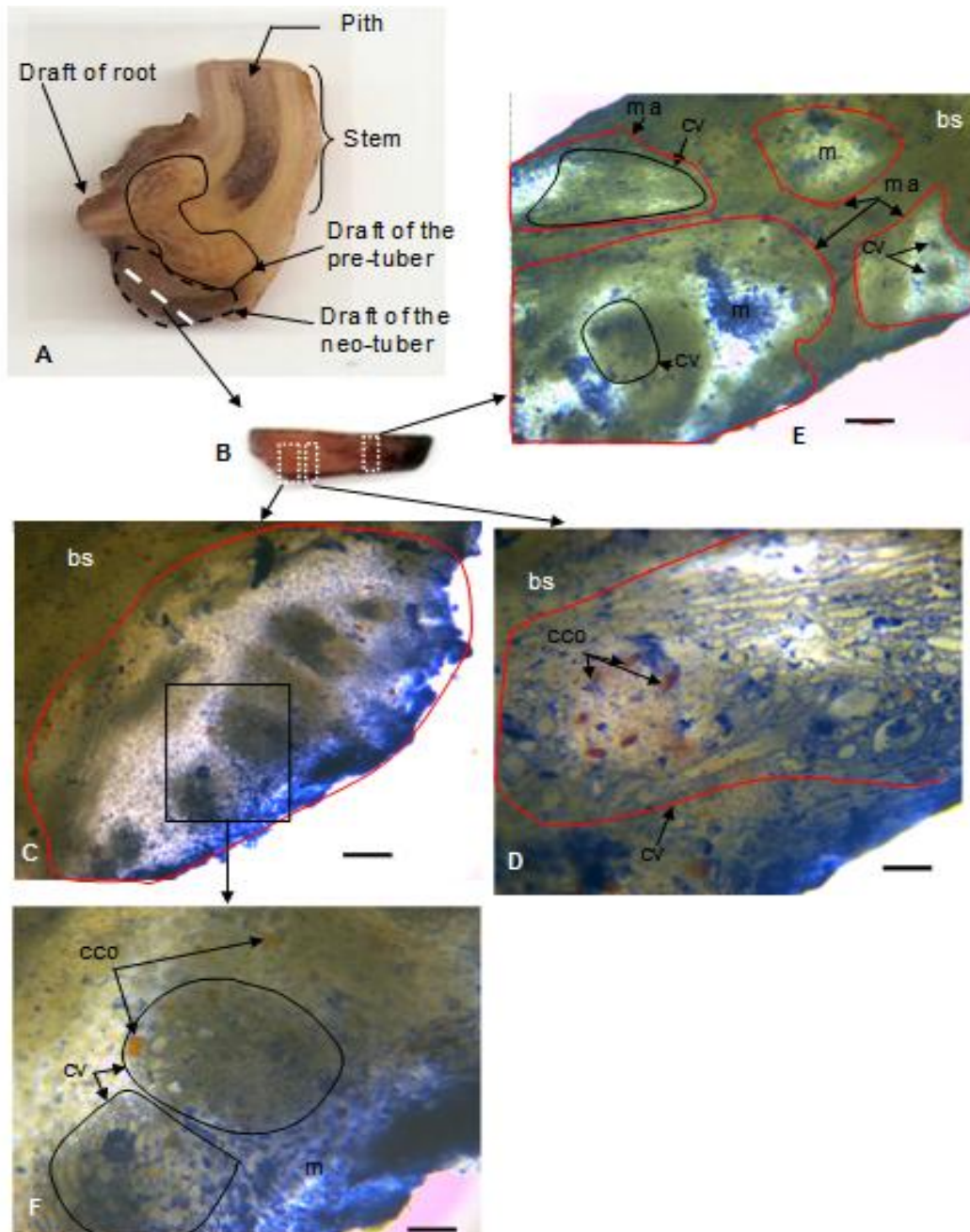


Fig. 3. Cross cutting of the seven-day-old of yam tuber of the kponan variety. (A) Longitudinal cutting of the tuber draft with a stem and a root. (B) Cross section sample. (C) Meristem area of the neo-tuber (G X 40). (D) Bloc of conductive vessels (g x 100). (E) Pocket of meristematic activity around the base of the stem (G x 40). The dashed line in A indicates the direction of the cut. The dotted squares in B indicate the observed parts. bs, base of the stem; m, meristem; ma, meristem area; cv, conductive vessels; cco, crystal of calcium oxalate. Scale bars: 25 μ m

Meristemic cell activity appears around the woody massif at the base of the stem. At inner of each meristem zone, the islets of vascular tissue are perceptible. These zones dislocate the

compact structure of the stem which will disappear for the emerging tuber (Fig. 3E). A mass of conducting vessels with different form was observed in the compact bloc of the basal stem (Fig. 3D).

The longitudinal cut stained by red congo ammoniacal highlights the procambial activity of the ligneous mass which leads to the setting up of the first xylem and phloem conductive elements (Fig. 4B). The cell mass tends to dislocate as we move away from the base of the stem to the benefit of young vessels. The meristem cells located between conductive tissues intensified their division activity in a centrifugal direction (Fig. 4E). The reconstituted image shows from the stem base to the lower end three parts. The fibrous part which forms the base of the stem is made of conductive vessels and parenchyma cells (Figs. 4C, D). Below this zone, the medullary parenchyma consists of a draft of more or less individualized conductive tissues and cells with meristematic activity. After this, a layer of generating base is observed. The third one is the cortical parenchyma with meristematic cells. On the tangential longitudinal plane, conductive vessels with well-individualized root-wall punctuation are also observed (Fig. 4C). After the first tissues are put in place, the latter will evolve and give the new tuber. The observations will therefore focus on the 30-day tuber that allows from the apex toward the proximal part to have a good view of the tissue evolution and the size of the starch grains.

3.1.3 Thirty days

At this stage, the tuber was divided into three equal parts (proximal, middle and distal) as indicated in methods, then a serial section from the apex toward the proximal part has been done. The longitudinal slice of the distal portion stained with iodine coloration showed a blue-violet coloration at both extremities (Fig. 5A). The reconstitution of the cut realized at 5 mm from the lower end (Figs. 5B, D) shows the generating base (procambium) in the form of an arc; Thick and close to the center of the tuber. The orientation of the arc is centrifugal indicating the direction of growth in thickness of the tuber. At this stage, the bark is more important compared to the marrow.

The procambial stained by the Lugol reagent parts the medullary parenchyma from the cortical

one (Fig. 5C). The two parts are differently composed. The first one is constituted by the draft conductive vessels and parenchyma cells. The cortical part was extended and generally formed by the parenchyma cells, the oxalate crystals and the epidermis. The cells of the generating base contain spherical vacuoles stained by the Lugol reagent. As the cuts move toward the upper end (10 mm of the lower end) of the distal portion, the thickening of the cambial arc decreases as well as the bark. The lower part of the cambial layer becomes important with a considerable number of conductive vessels (Fig. 5D). The future conductive vessels have a thinness form more important near the cambium and rounded inner the medulla parenchyma.

At 15 mm from the lower end of the apex, (Fig. 6A) the curvature of generating base decreases as well as the intensity of staining by the Lugol (Fig. 6B). The different tissues of the bark begin to differentiate. From the extremity to the generating base, a layer of desquamated cell was observed, followed by three layers of brown-coloured suberified cells of radial tangential orientation (Fig. 6H). At these three cellular bases, there are four others arranged in the same way, and with a vacuole. As the tuber approaches maturity, the suberification will gain the other sub-adjacent cells with a reduction of cortical parenchyma. The number of suberized layers is variable because the epidermis has a contour in sawtooth. Under epidermis, a zone of heterogeneous cells according to their contents was observed. Some of them content colored substance by the lugol reagent in yellow (Fig. 6C, I). A large zone of vacuolated cells colored by the Marshall Red ammoniacal was observed under the heterogeneous one and contiguous to the generating base (Fig. 6B, C). This area is characterized by an important content of cells in vacuole (Fig. 6G) and the evolution of these cells is oriented toward the epidermis. From the generating base to the tuber marrow, near the procambium, an analogous evolution of cells oriented toward the inner was observed (Fig. 6D). Cell development around this area was oriented on both sides of the generating base represented by a line. After that, medullary parenchyma composed by future conducting vessels (xylem and phloem) and amyloplast (Fig. 6B, E, F) was observed. At this stage, starch granules are not well observable.

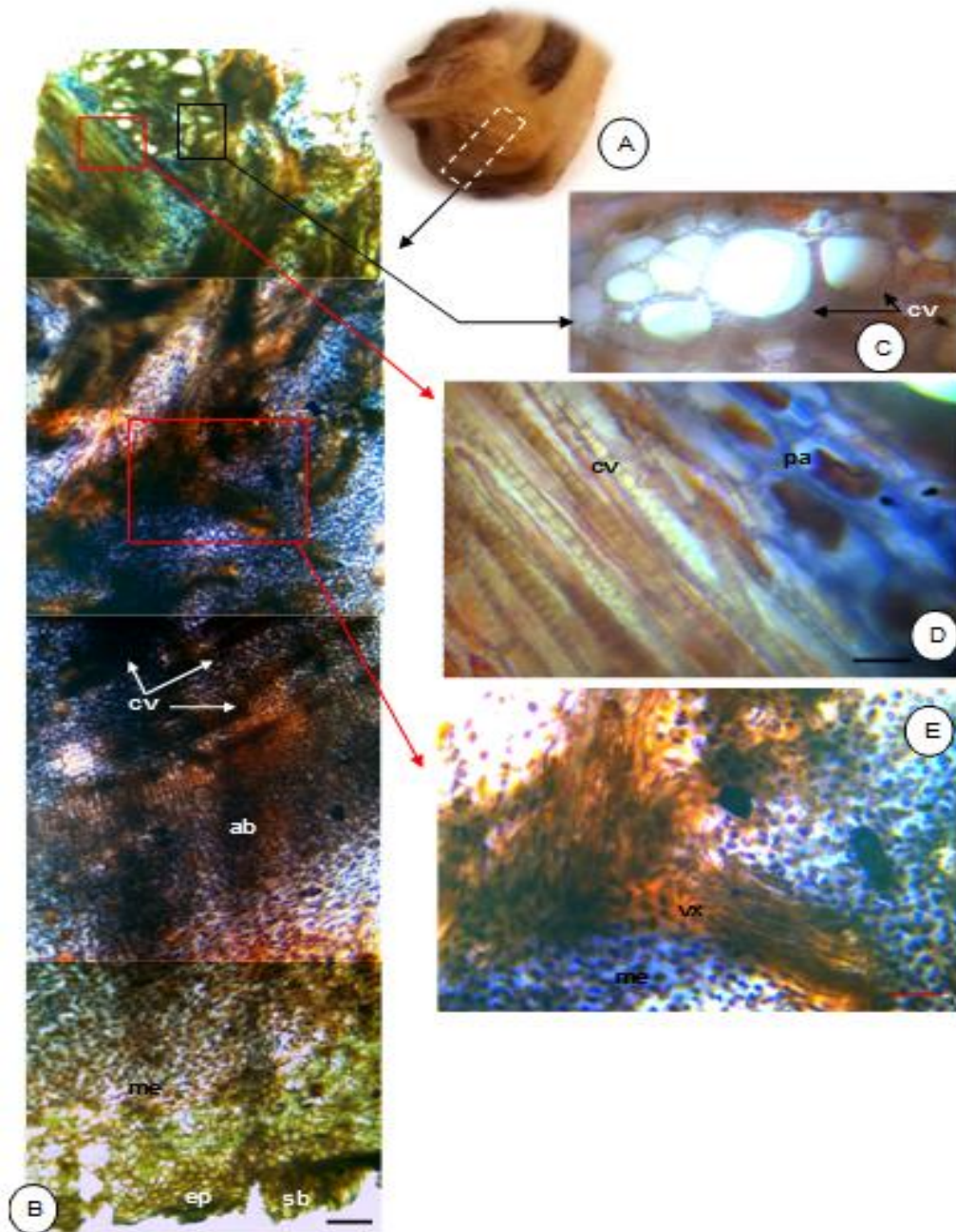


Fig. 4. Longitudinal section of seven-day-old yam tuber of the kponan variety stained with Marshall Red ammoniacal. (A) Tuber draft indicating the cut made. (B) Reconstitution of the cut (Gr X 40). (C, D (Gr X 400) and E (Gr X 100)) Conductive vessels. ab, generating base ; ep, épidermis ; pa, parenchyma ; me, meristem; sb, suber; cv; conductive vessels. Scale bars: 25 µm

As the cuts approach the middle part, more precisely at the lower end of this part, the meristematic character of the generating base tends to disappear (Fig. 7). The parenchyma

cells were more stained by the lugol with an intense coloration around the conducting vessels (Fig. 7B). Starch granules with different form arranged on the cell wall were observed

(Fig. 7D, G). Toward upper end of the middle tuber part, conducting vessels differentiated, present a resorbed cytoplasm (Fig. 7E). The xylem with a striped, punctuated and thickened cell wall differed clearly from the phloem (Fig. 7F). Cell wall thickening was observed on cortical parenchyma for a section realized at upper extremity of proximal part of the tuber (Fig. 8C). Some of them content starch granules

stained orange yellow by the Marshall Red ammoniacal. The cells of the medullary parenchyma are more filled with starch grains whose size increases and variable form (Fig. 8E). From distal part toward the proximal one, were observed: 1.26 μm to 3.53 μm (distal part), 4.49 μm to 5.86 μm (for middle one) and 6.06 μm to 8.36 μm for the proximal part.

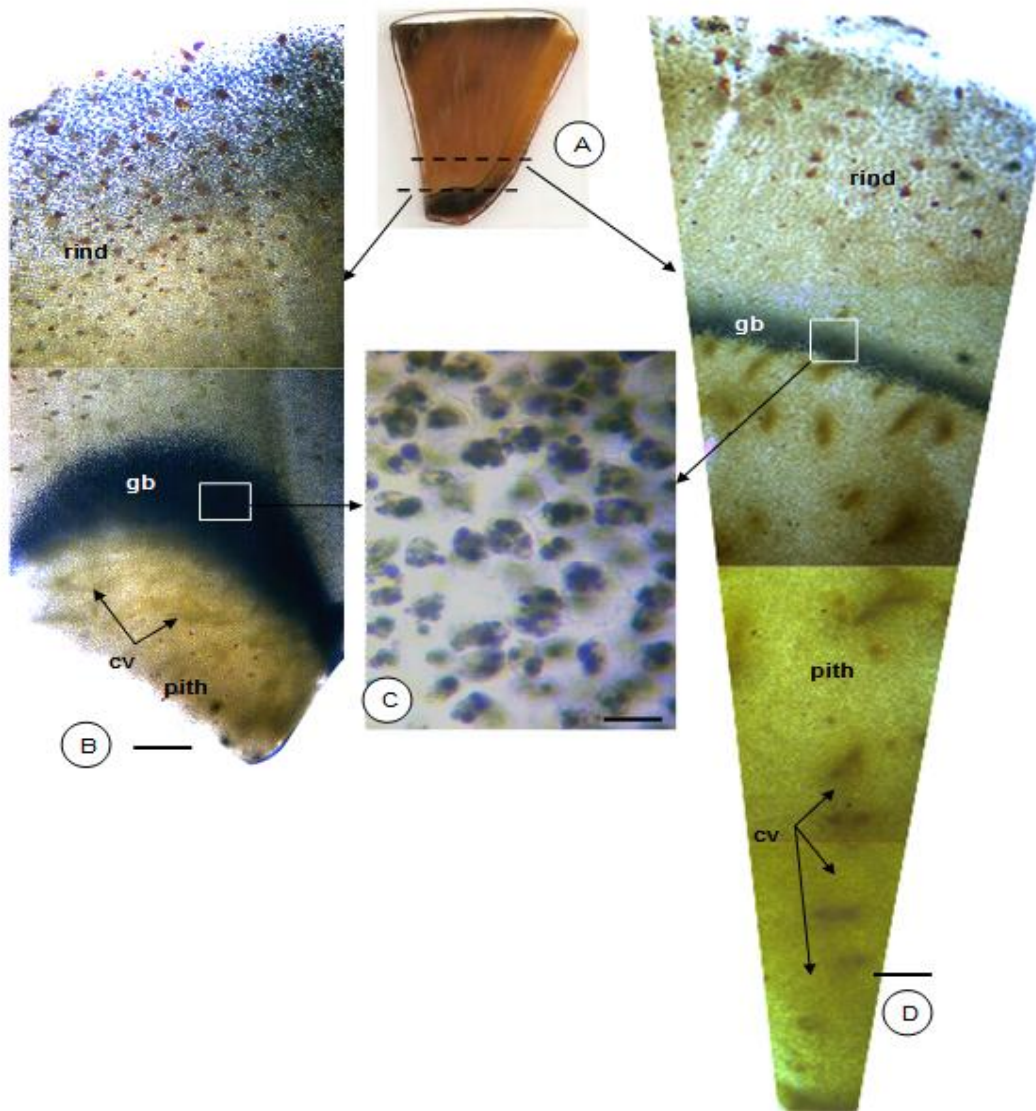


Fig. 5. Cross sections made from the apical end of the distal part of the Yam tuber stained by the lugol reagent var. kponan. (A) The quarter of the distal part. (B, D) cut at 5 mm and 10 mm respectively from the lower end (Gr x 40). The conductive vessels (cv) are arranged in the lower part of the generating base (gb). The seat separates the bark from the pith. The closer the cuts are to the upper end, the bark size decreases as well as the thickness of the generating base. Scale bars: 25 μm

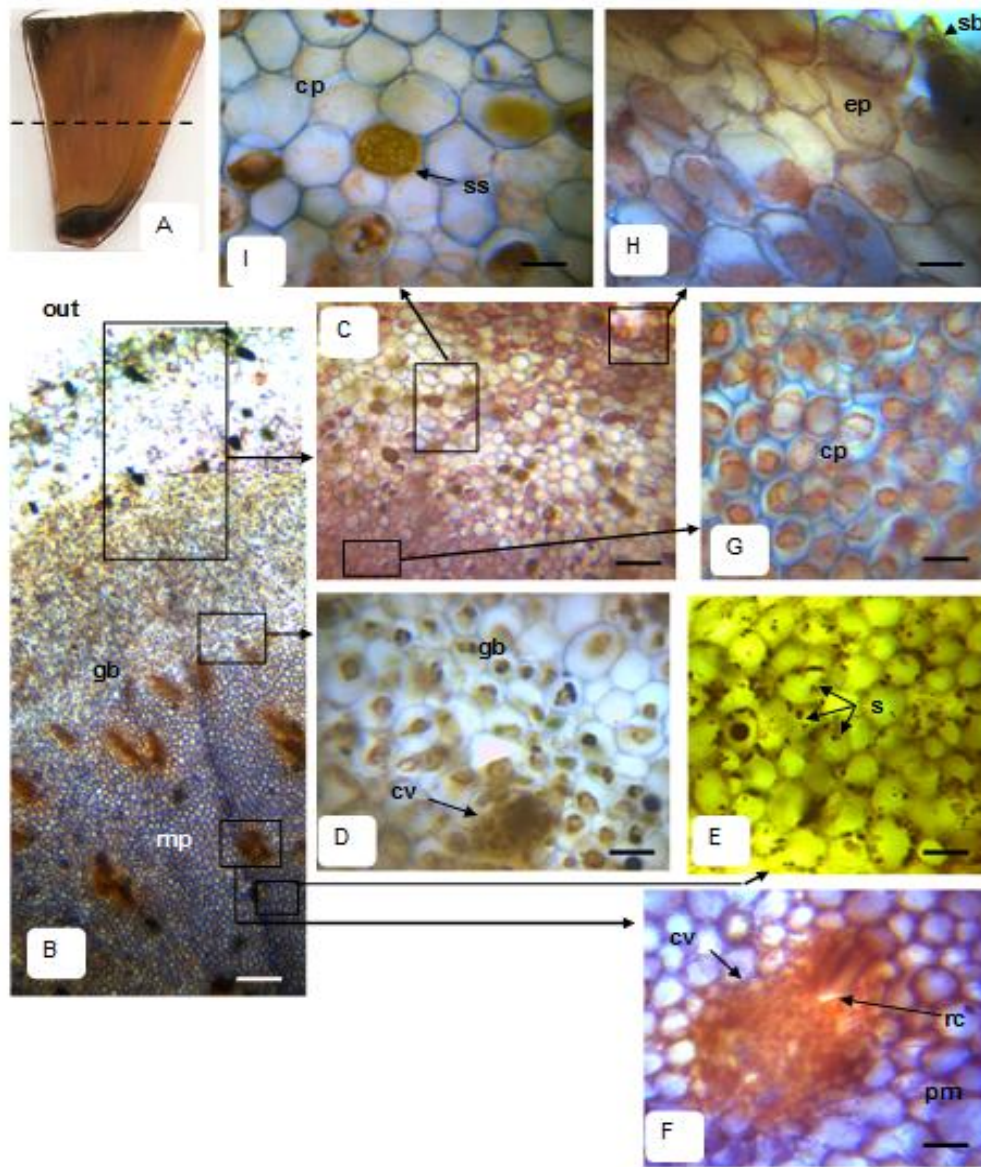


Fig. 6. Cross section made at 15 mm from the lower end of the distal part (var. kponan). (A) The quarter of the distal part. (B) Reconstitution of the cut (Gr X 40). (C) Cortical parenchyma (Gr X 100). (D) Generating base or procambium (Gr X 400). (E) Draft of amyloplast stained by the lugol (Gr X 400). (F) Draft of conductive vessel with resorbed cytoplasm (rc) (Gr X 400). (G) Cortical parenchyma with colored substance (Gr X 400). (H) From epidermis (ep) to the medullar parenchyma (mp), were observed the suberosus area (sb) with five to six cells layers. Below, the cortical parenchyma (cp) made up cells with solid substance (ss) stained yellow by the lugol or not and vacuolated ones with meristematic activity near the generating base (gb). Below the procambium, the medullar parenchyma with undifferentiated conductive vessels (cv) and draft of amyloplast containing starch (s). Scale bars: 25 μ m

These starch grains observed in white light (Fig. 8F) have variable shapes (spherical, ellipsoidal and ovotriangular). Growth streaks are observable on certain grains. Certain conducting

vessels observed in the distal and middle parts remain undifferentiated (Fig. 8D). It were presented as a stacking of bundle and not stained by the lugol reagent.

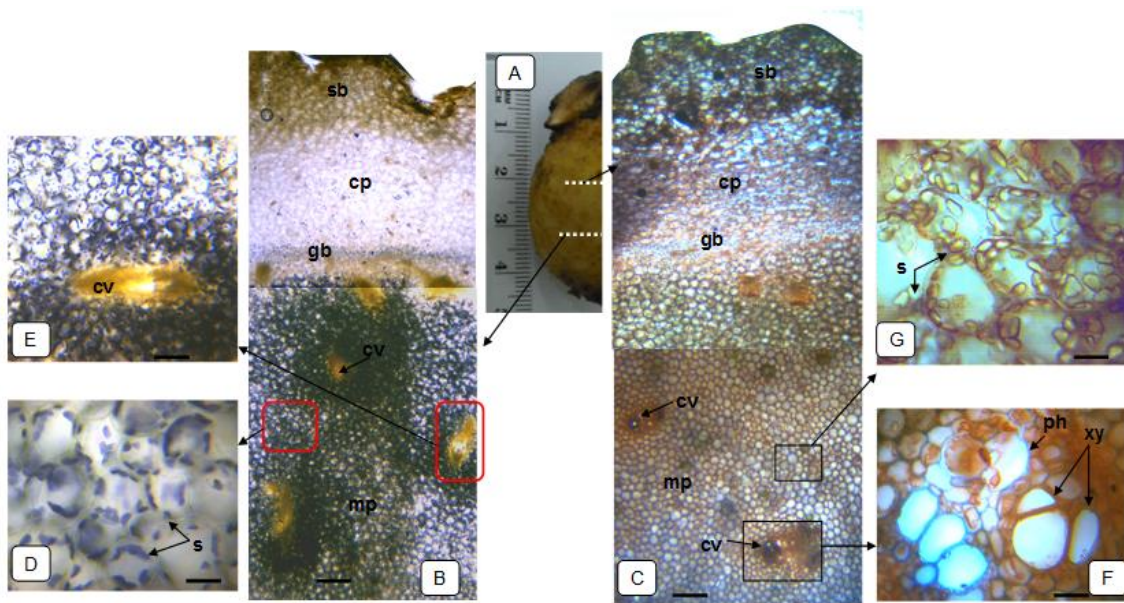


Fig. 7. Cross section made at both ends of the middle part (var. kponan). (A) Tuber of 30 days of kponan variety. (B) Reconstituted cut of the lower end of the medial section (Gr X 40). (C) Reconstituted cut of the upper extremity of the median part (Gr X 40). (D, G) Amyloplast containing starch grains (Gr X 400). (E, F) Conductive vessels (Gr X 400). cv, conductive vessel; mp, medullar parenchyma; cp, cortical parenchyma; gb, generating base; xy, xylem; ph, phloem; s, starch. Scale bars: 25 μm

The tangential section (Fig. 9) reconstituted presents the two great parts separated by an annular generating cell layer as in cross section. The inner part to the cell layer is constituted of draft of conductive vessels and the outer part rich in calcium oxalate crystals. Lugol staining (Fig. 9B) shows no evidence of starch grains presence apart from the cambium cell layer which the vacuole is colored dark blue. These very vacuolated cells take a centrifugal orientation on either side of the generating base (Fig. 9D, H). The lower part of the procambium (towards the apex) is constituted of cells without particular orientation with irregular shape and size. It formed an aggregate of cutinized cells surmounted by a coif (Fig. 9C, F, G). The cells at inner (medullar parenchyma) the section are arranged in line along the longitudinal axis (Fig. 9E). They are very close and vacuolated. Similarly, the conducting vessels are also arranged in line (Fig. 9I).

As has been observed at different level of cross sections, here also inner border of the cambium is constituted of a conducting vessel generally of rounded form. Similarly, the outer part of the generating layer is made up of three distinct parts due to the intensity of the lugol coloration.

A clear area Contiguous to the procambium followed by a little dark very rich in crystals of calcium oxalate all topped by the coif was observed. The cells of the coif are less joined, leaving spaces between them. Those of the meristem do not show any intercellular space. The more enlarged observation shows a difference between the shapes of the cells. Those located between the coif and the procambium, have a shape of isodiametric nature or rounded without particular orientation. At the lower part of the generating area, the cells are rectangular in shape and aligned according to the longitudinal plane with no meatus (Fig. 9E, I).

3.2 Scanning Microscopy Observation of Yam Tuber at Thirty Days after Tuberization

Direct observations without metallization to gold powder by scanning electron microscope did not allow the yam tissue to be observed at the beginning of tuberization. Samples of young yam tissues that are not rolled to gold powder tend to deform during observation. Thus, a sample of three (3) months after the start of the Tuberization was selected. The three-

dimensional observation shows that the cells are generally of ovosphérique form with tapered end (Fig. 10A, B). They are more like oval-shaped baskets suitable for filling by starch grains. This dimpled structure of irregular diameter also has the allure of a honeycomb. The lower part of the distal area shows no traces of starch grains (Fig. 10A, B). But as the sections approach the upper end, the first grains of starch appear around the conductive vessels (Fig. 10C). The filling of the amyloplasts extends to the rest of the cells of the

medullar parenchyma. This gives the appearance of a basket full of grains (Fig. 10D). These starch grains generally have an oval triangular shape flattened in the longitudinal direction of the triangle (Fig. 10F). The observed sample seems to show the only form. The conductive vessels at this stage of tuber growth exhibit a completely resorbed cytoplasm giving the appearance of a pipe (Fig. 10E). The wall of these conductive elements is perforated giving the appearance of a net (punctuation).

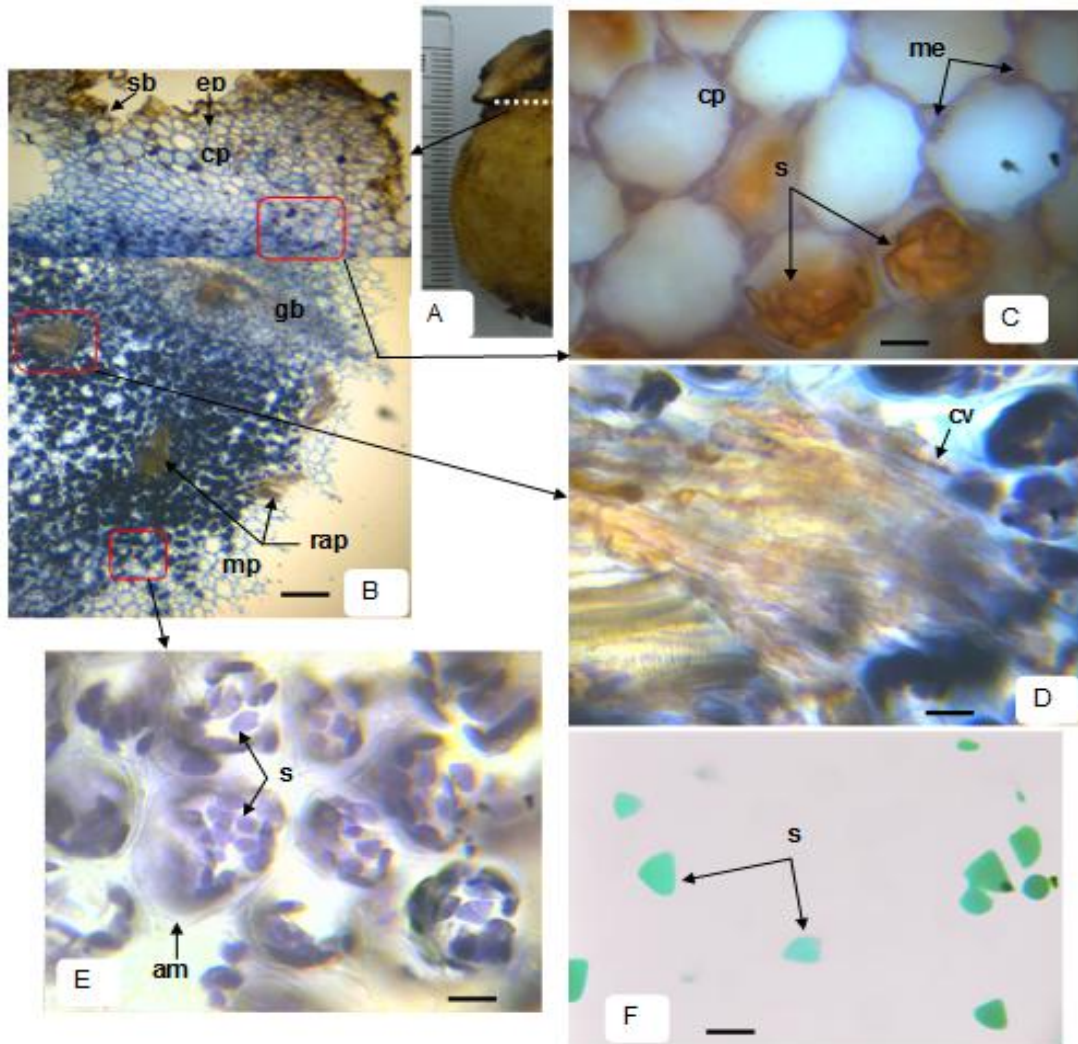


Fig. 8. Cross section made at the upper end of the proximal part. (A) Tuber of 30 days of kponan variety. (B) Partial reconstitution of the cut (Gr : x 40). (C) Cortical parenchyma consisting of a few amyloplasts (Gr X 400). These thick-walled cells arrange space between them or meat (me). (D) Undifferentiated conductive vessel (Gr X 400). (E) Amyloplast containing starch (Gr X 400). (F) Starch granules (Gr X 400). am, amyloplast; cp, cortical parenchyma; cv, conducting vessel; ep, epidermis; gb, generating base; me, meat; mp, medullar parenchyma; s, starch; sb, suber. Scale bars: 25 µm

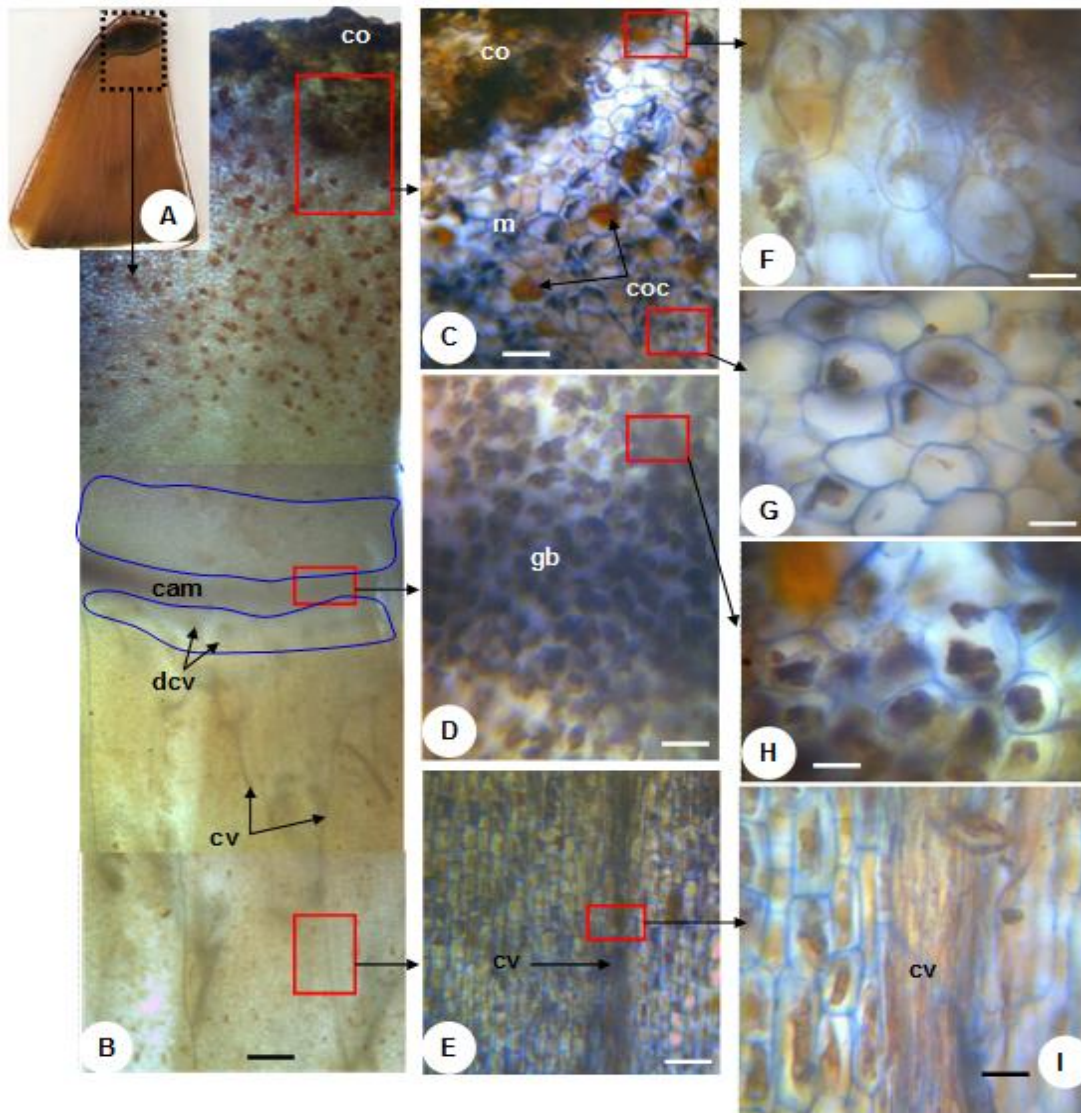


Fig. 9. Longitudinal section at the lower end of the distal part. (A) Portion of observed apex. (B) Reconstitution of the colored cut with lugol (Gr X 40). (C) Portion of the cortical parenchyma (Gr X 40). (D) procambium or generating base (Gr X 40). (E) Medullar parenchyma with conductive vessel (Gr X h40). (F) Cells of the coif (Gr X 400). (G) Cortical parenchyma (Gr X 400). (H) Cambial cell with colored vacuole (Gr X 400). (I) Medullar parenchyma (Gr X 400). The generating base (gb) is surrounded by a clear zone of different composition. The lower part contains draft of conductive vessel (dcv) and the upper part, meristem cells (m). The epidermis is covered with a coif (co). cam, cambium; coc, crystal oxalate calcium; cv, conductive vessel. Scale bars: 25 μ m

The beginning of yam tuberization results from a protuberance of the stem base due to the activity of the meristematic cells, followed by the placement of the first tissues. The cambial activity ensures the growth in length and width of the tuber (Fig. 11). The activity of the meristematic cells located at the apex actively

participated in the development of the tuber. The cambial activity of the generating base decreases from the distal to the proximal end. This activity responsible for the longitudinal and radial growth of the tuber ceases at maturity of the tuber.

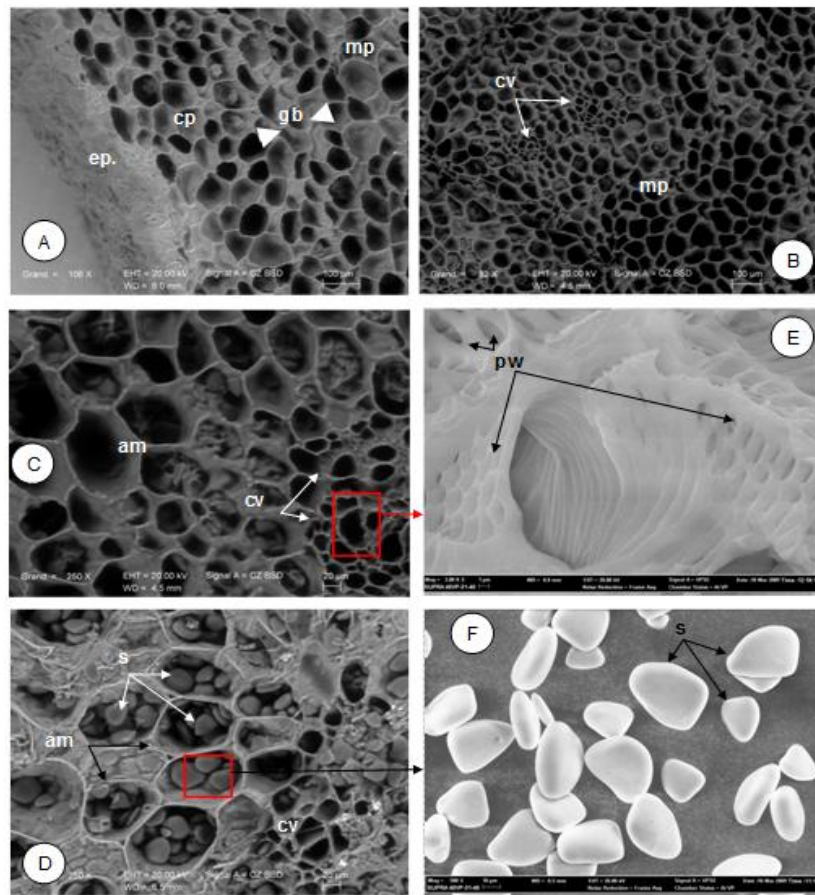


Fig. 10. SEM observation of a cross-section at the lower end of the middle portion of a sample of three months after the beginning of tuberization (kangba variety). (A) Cortical parenchyma. (B) Medullar parenchyma. (C) Conductive vessel. (D) Amyloplast. (E) Punctured wall of xylem. (F) Starch. am, amyloplast; cp, cortical parenchyma; cv, conductive vessel; ep, epidermis; mp, medullar parenchyma; pw, punctured wall; s, starch

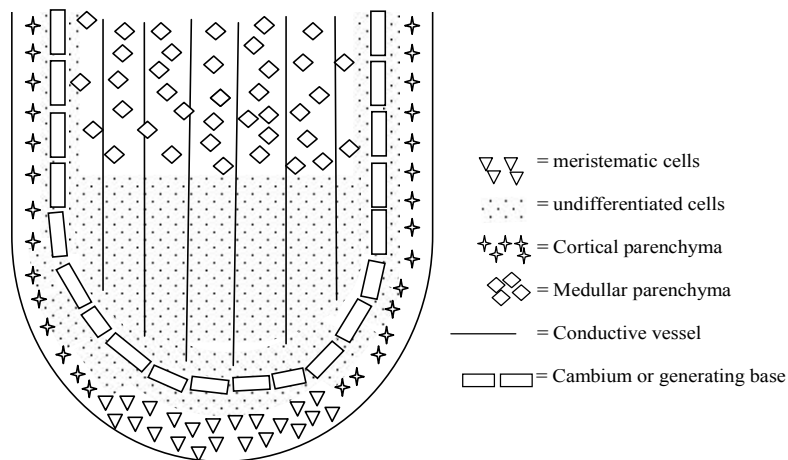


Fig. 11. Diagram of a longitudinal section showing the growth pattern of the yam tuber *D. Cayenensis-rotundata*, kponan variety

4. DISCUSSION

The origin of the yam tuber's birth has been controversial for decades. Sometimes it emanates from a main root, or from the first between the stem node and thus is part of the hypocotylaires bulbs, or an intermediate zone between the stem and the root. The work of Sealy (e. g., HL. Sealy 1982. Thesis, Univ. of Nantes, France) on the histological study of the phenomenon of hardening of the yam tuber of *D. Dumetorum* after harvest, showed a structure of shoot type of the tuber, confirming this study. Indeed, the scanned radial cut at the beginning of the tuberization clearly shows that the neo-tuber emanates directly from the stem base. The intersection between the stem and the neo-tuber composed of lignified fibers is a cluster of dead cells [13]. It will later be the pre-tuber. The light microscope observation also shows a bulge in the lower end of the stem base indicating the origin of the yam tuberous stem. The same observation was made in the potato. According to [14], the tuber is not a modification of the root but of the stem. Histologically, the origin of the tuber appears to emanate from two different types of cells, constitutive of the stem base: the ligneous parenchyma or Sclerenchyma and the perivascular cambium. The ligneous parenchyma may at some point return to a meristematic state and generate other cells whose future differentiation will yield the different tissues of the tuber [13]. The cambium supplies mainly to the interior of the reserve tissue and collateral vascular bundles.

At the cross-sectional level, the generating base is more active at the apex. This is explained by the intensity of the coloration at the Lugol. But the closer the cuts are to the median part, this activity would stop. Indeed, the cells of the cambium with a meristematic activity would be composed of numerous vacuoles whose contents are coloured by the Lugol. More the cells differentiate; more or less the number of spherules is reduced to 1 or 2, depending on the cell's destiny [13]. The amyloplasts cells would have a small vacuole. As the growth of the tuber continues, the cells differentiated. Thus, the medullary parenchyma is formed of starch reserve tissues. The cytoplasm of conducting vessels is resorbed to facilitate the transit of elaborated substances and of synthesis [13]. The cortical parenchyma is enriched in calcium oxalate crystals of varying geometric form. Serial cuts carried out according to the transverse plane on distal part of the tuber harvested at 30

days after the initiation of tuberization, indicate the existence of a gradient of maturity of the tuber. This results in the placing of the starch that is made from 2 cm from the apex as well as the thickening of the cell wall. Indeed, in the case of tuber length growth, cell formation occurs at the level of the active zone observed on either side of the cambium in the longitudinal plane. Once formed at the apical meristem, these cells differ a little more towards the medial part of the tuber and constitute the different tissues of the yam tuber. The sense of this differentiation is centripetal. This phenomenon is responsible for the elongation of the tuber. This growth gradient was revealed by the study of the morphogenesis of Tuberization performed by Trouslot [9]. But as the tuber approaches maturity, growth slows and ends in a month of senescence always according to the same source. This results in a decrease in the proportion of undifferentiated tissues. The meristem activity of the cambium would then be reduced. For thickness growth of yam tuber, this could still be a "cambiale" activity of the generating base that would be the origin. In fact, cross sections showed that on both sides of the generating base, a layer of highly vacuolate cells emanating directly from it was observed. This production of cells whose differentiation would give the cortical parenchyma on the outer side of the generating base and the medullary parenchyma on the inner side would cause growth in tuber thickness. This particular type of cambial tissue exists in some Liliaceae and Dioscoraceae [13]. According to Lawton and Lawton [15], in elongated tuber species, a highly meristematic apex develops; the activity at a certain distance from the summit would cease. In spherical tuber species, the meristem would remain active on the entire surface of the tuber until maturity. This was observed in this study.

5. CONCLUSION

The histological study of the onset of tuberization revealed the existence of a longitudinal growth gradient whose point of growth is the apex and the sense of maturity of the distal part at the proximal end. The cambium generates cells at the lower end of the apex. These very vacuolated cells differentiate and will form the median and proximal parts of the tuber. This cambium generating activity would cease when the tuber become matures. The distal part will remain immature compared to the other two parts. This explains the variations observed in the cooking of the tuber and its properties.

ACKNOWLEDGEMENTS

The authors wish to thank the international Foundation of Science (IFS) for funding this research (E4489-1) and the laboratory of oil research of PETROCI for making the SEM available.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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