

1 **Histological study of the polarity of yam (*Dioscorea spp.*) at**
2 **the beginning of tuberization**
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14 **ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)**
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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 are born 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 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.

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17 *Keywords: starch; D. Cayenensis-Rotundata, histology; yam tissue, polarity; Tuberization*
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24 1. INTRODUCTION

25 The yam is an annual plant whose vegetative cycle is related to the seasonal cycle. Although classified among
26 monocotyledons, the Dioscoreacea family has a number of characteristics that can be found in dicotyledons with, for
27 example, a cauline apical apex of the embryo (H. L. Sealy 1982. Thesis, Univ. of Nantes, France). Tuber germination is
28 associated to the first external indication of renewed growth was the development of small protuberances on tuber surface
29 [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*
30 *rotundata*. The number of stems varies according to species and their size can reach 30 m of height especially in wild
31 species [2]. The tuber, of the Latin Tuberculum, is a bulging caulinary organ, usually underground belonging to a vascular
32 plant and at which the plant stores reserves. Sometimes it is the cortical parenchyma that becomes hypertrophic and
33 serves as a warehouse, sometimes it is the medullary parenchyma. In fact, because of this capacity to store reserves,
34 tubers play a nutritional role for both plants and animals [2]. Yam serves as an important source of carbohydrate (83.08%
35 to 86.13%) and serves as a major source of income in countries where they are cultivated [3, 4, 5]. The world production
36 of yam is widely dominated by West Africa with more than 92% [6]. In terms of production, green manure such as
37 intercropping with pigeon pea improves quality and yield of tuber [7]. The technique of pre-treatment of seed by pesticides
38 (fungicide + insecticide) improves germination rate and tuber yields [8].

39 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-
40 tuber, called complex bud, is an unconsumable fibrous mass, from which the different organs of the new plant develop.
41 The neo-tuber or tuber mass has three parts, the proximal zone of which is directly connected to the pre-tuber and
42 represents the head of the tuber. The second part of the tuber (middle) is an extension of the proximal part and third, the
43 distal part which is the growth zone of the neo-tuber [9].

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

55 2. MATERIAL AND METHODS

57 2.1. Experiment field

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

62 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
63 carried out in 2013-2014, was submitted to the climate of bass cost. The mean climatic parameter is: temperature

(36,9°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

(H. L. Sealy 1982. Thesis, Univ. of Nantes, France). Small cubes of yam tissue were cut into sections of 15 μm 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.

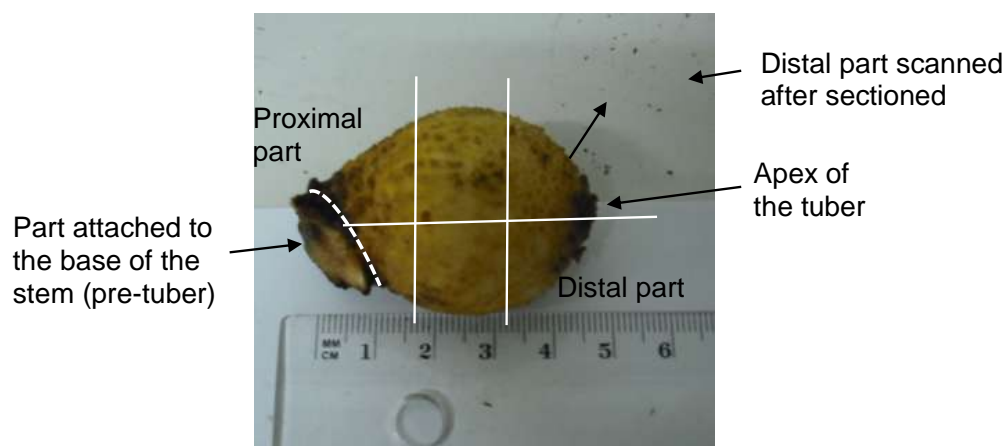


Figure 1: Technique for cutting and sampling yam tissue for histological study.

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

92 with Lugol, rinsed, then stained with Congo red and revealed with sulphuric acid. This makes it possible to observe both
93 the membrane and the cellular content.

94 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)
95 prepared according to Locquin and Langeron [12]. This reagent stains starch in blue. After the Microtome cutting step, the
96 sample is collected in water, washed for 5 min, stained with Lugol for 3 min, and then washed in water before being
97 observed. The mounting medium is distilled water.

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

101 **2.3. Scanning electron microscopy**

102 The size of the samples fixed to formol-calcium was first reduced (0.3 mm x 0.3 mm) under a binocular magnifying lens
103 before being dehydrated. The dehydration is carried out by successive passage in 4 ethanol baths of increasing degree
104 (70°GL, 80°GL, 90°GL and 100°GL) followed by 2 acetone baths (H. L. Sealy 1982. Thesis, Univ. of Nantes, France). The
105 microscope used is of the brand Zeiss (SWPRA 40, 3148, Oberkochen, Germany). As a special feature of this apparatus,
106 the samples do not need to be covered with a thin gold film as was the case with previous models. Thus, after
107 dehydration, the samples are mounted directly on the object plots and observed with a scanning electron microscope. The
108 image was analysis by the software INCA drycool, Oxford.

109 **3. RESULTS**

111 **3.1. Light microscopy**

112 **3.1.1. Tuber initiation**

113 The bursting stage of the suber marks the beginning of tuberization. The cross-section (Figs. 2A, B) shows the anatomical
114 structure of the visual initiation of tuberization. The apical end of the stem initiates an outgrowth that spreads around the
115 stem. From the outside to the inside, two areas stand out. The first part stained blue by lugol reagent consists of the
116 meristem cells whose division activity results of the growth of the neo-tuber. The second area coloured yellow by the lugol
117 consists of a draft of conductive tissue oriented in the direction of tuber growth and the third one corresponds to the
118 fibrous inner part of the pre-tuber. It is difficult to identify the cell wall of the meristematic cells as well as the conductive
119 elements, because the cut is thick. Calcium oxalate crystals in an orange-coloured are observed on the entire surface of
120 the section.

121 **3.1.1. Seven days after tuber initiation**

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

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

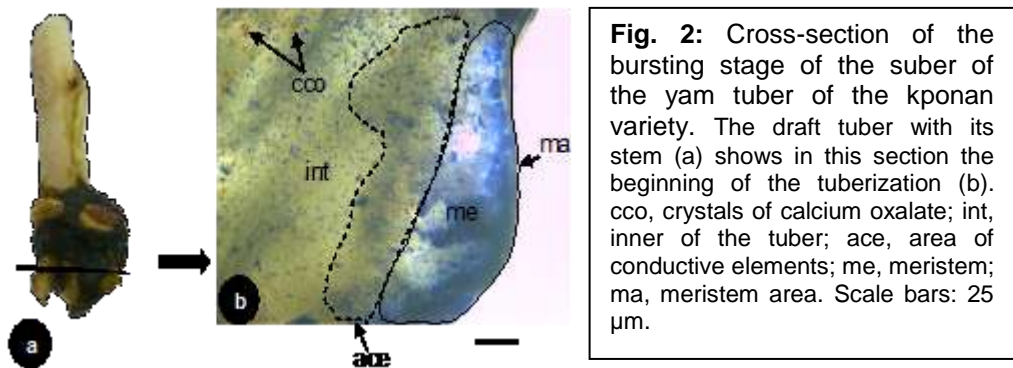
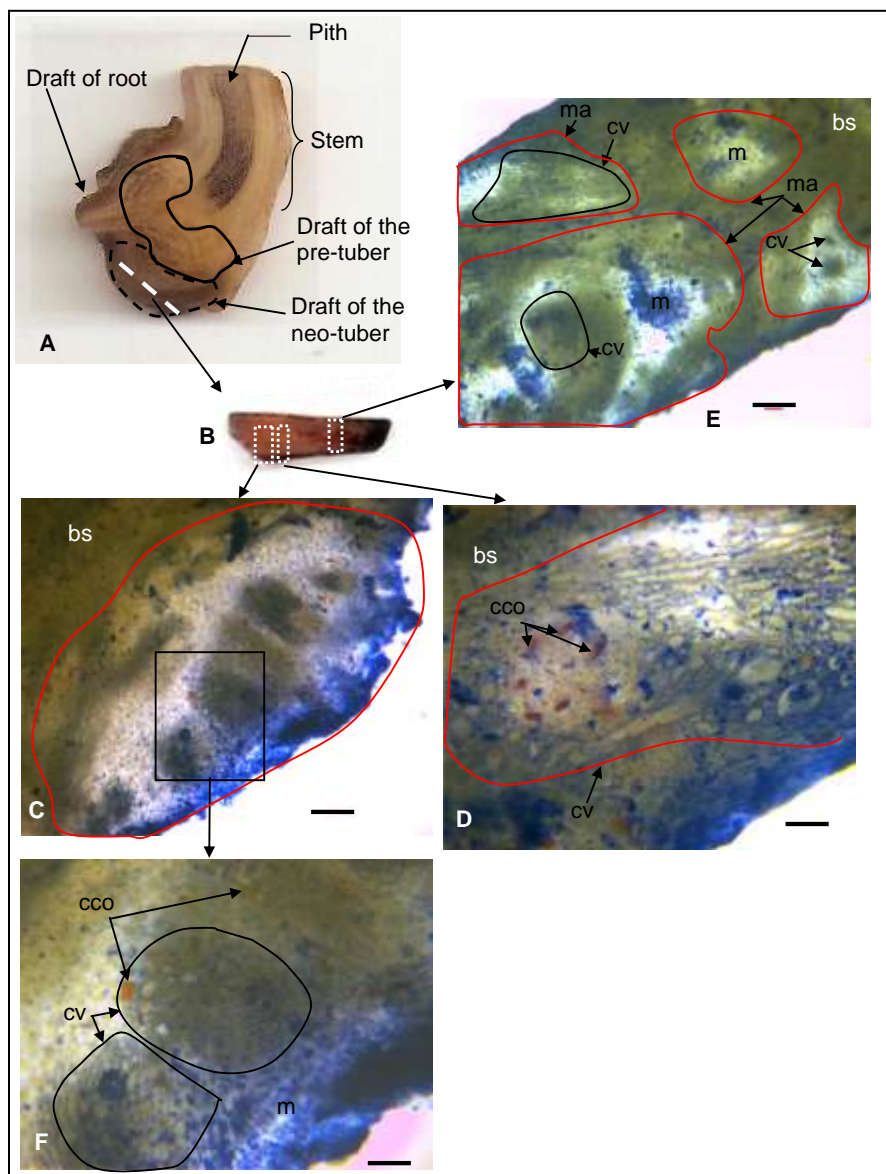


Fig. 2: Cross-section of the bursting stage of the suber of the yam tuber of the kponan variety. The draft tuber with its stem (a) shows in this section the beginning of the tuberization (b). cco, crystals of calcium oxalate; int, inner of the tuber; ace, area of conductive elements; me, meristem; ma,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

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Figure 3: Cross cutting of the seven-day-old of yam tuber of the kponan variety.

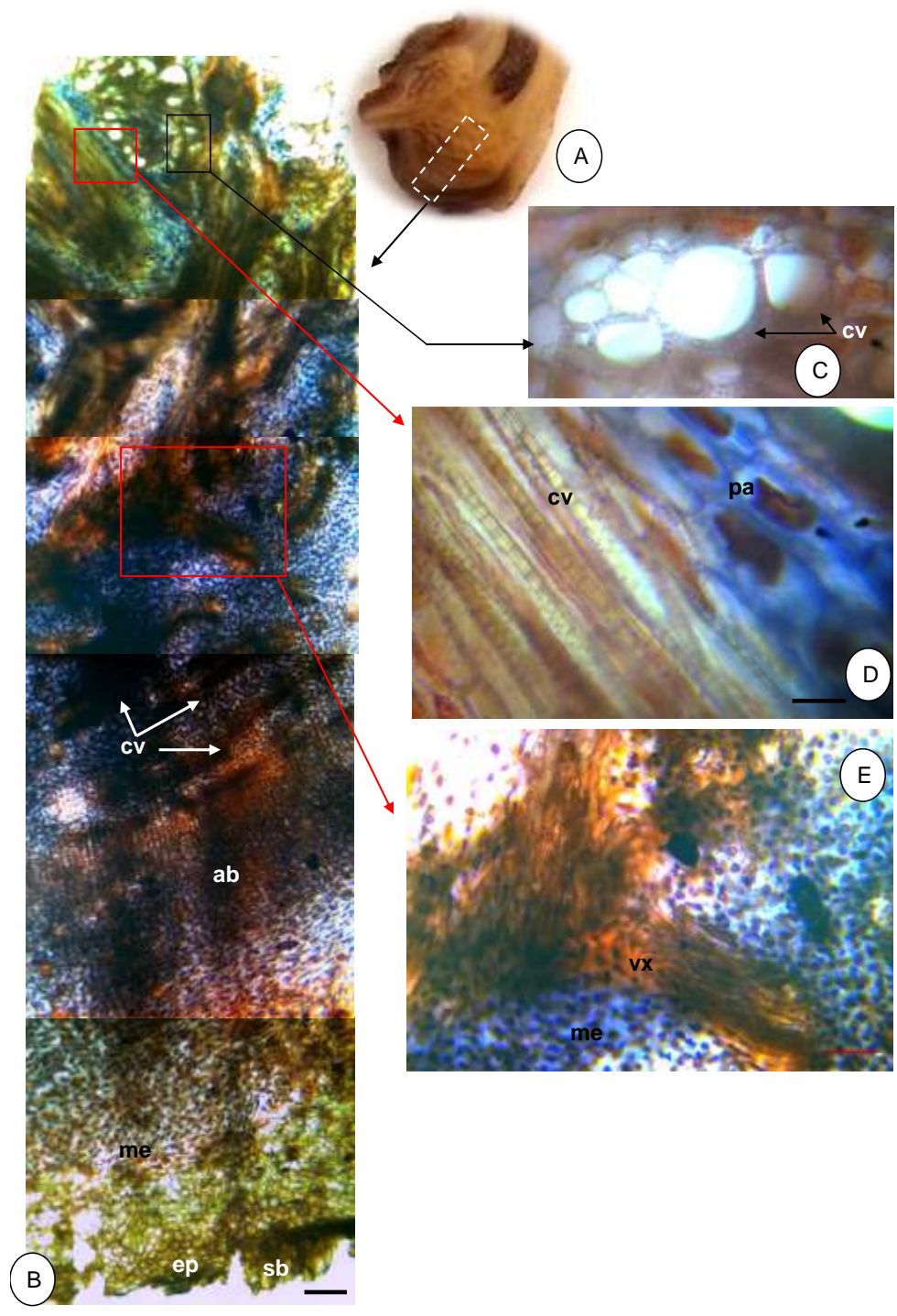
(A) Longitudinal cutting of the tuber draft with a stem and a root. (B) Cross section sample. (C) Meristem area of the neo-tuber (G X 40). (D) Bloc of conductive vessels (g x 100). (E) Pocket of meristematic activity around the base of the stem (G x 40). The dashed line in A indicates the direction of 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.

140 Meristemic cell activity appears around the woody massif at the base of the stem. At inner of each meristem zone, the
141 islets of vascular tissue are perceptible. Theses zone dislocat the compact structure of the stem which will disappeared for
142 the emerging tuber (Fig. 3E). A massif of conducting vessels with different form was observed in the compact bloc of the
143 basal stem (Fig. 3D).

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

156 **3.1.2. Thirty days**

157 At this stage, the tuber was divided into three equal parts (proximal, middle and distal) as indicated in methods, then a
158 serial section from the apex toward the proximal part has been done. The longitudinal slice of the distal portion stained
159 with iodine coloration showed a blue-violet coloration at both extremities (Fig. 5A). The reconstitution of the cut realized at
160 5 mm from the lower end (Figs. 5B, D) shows the generating base (procambium) in the form of an arc; Thick and close to
161 the center of the tuber. The orientation of the arc is centrifugal indicating the direction of growth in thickness of the tuber.
162 At this stage, the bark is more important compared to the marrow.



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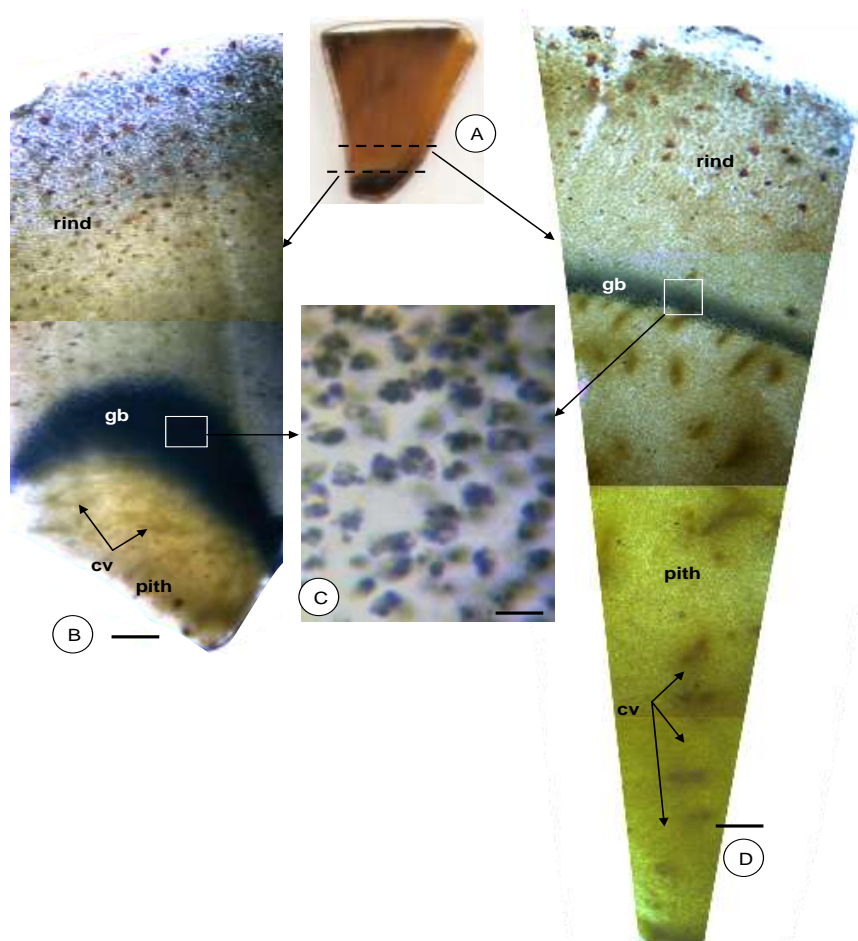
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Figure 4: Longitudinal section of seven-day-old yam tuber of the kponan variety stained with Marshall Red ammoniacal. (A)Tuber draft indicating the cut made. (B) Reconstitution of the cut (Gr X 40). (C, D (Gr X 400) and E (Gr X 100)) Conductive vessels.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

The procambial stained by the Lugol reagent parts the medullar 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

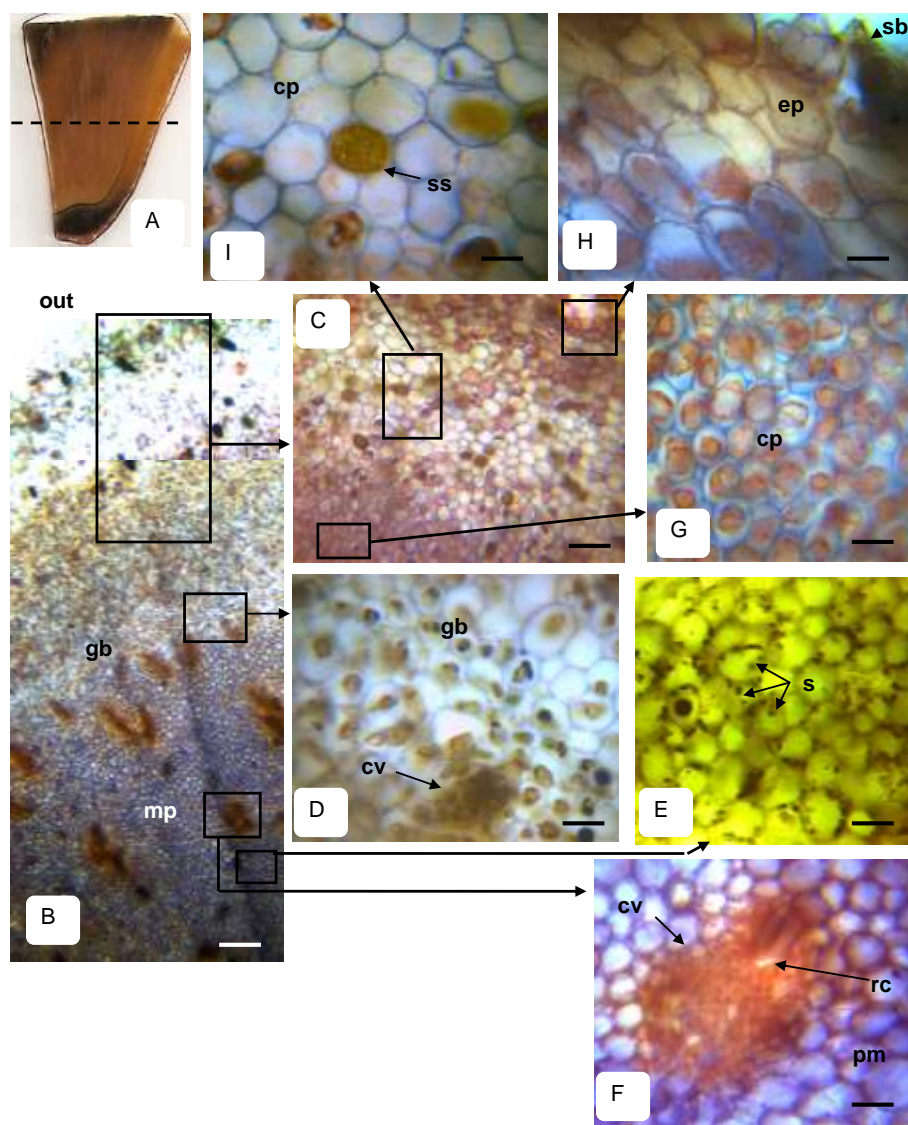
173 the cambial layer becomes important with a considerable number of conductive vessels (Fig 5D). The future conductive
 174 vessels have a thinness form more important near the cambium and rounded inner the medulla parenchyma.
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 177 **Fig. 5:** Cross sections made from the apical end of the distal part of the Yam tuber stained by the lugol reagent var.
 178 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
 179 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
 180 are to the upper end, the bark size decreases as well as the thickness of the generating base. Scale bars: 25 μ m.
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182 At 15 mm from the lower end of the apex, (Fig. 6A) the curvature of generating base decreases as well as the intensity of
 183 staining by the Lugol (Fig. 6B). The different tissues of the bark begin to differentiate. From the extremity to the generating
 184 base, a layer of desquamated cell was observed, followed by three layers of brown-coloured suberified cells of radial
 185 tangential orientation (Fig. 6H). At these three cellular bases, there are four others arranged in the same way, and with a
 186 vacuole. As the tuber approaches maturity, the subterification will gain the other sub-adjacent cells with a reduction of
 187 cortical parenchyma. The number of suberized layers is variable because the epidermis has a contour in sawtooth. Under
 188 epidermis, a zone of heterogeneous cells according to their contents was observed. Some of them content colored
 189 substance by the lugol reagent in yellow (Figs. 6C, I). A large zone of vacuolated cells colored by the Marshall Red
 190 ammoniacal was observed under the heterogeneous one and contiguous to the generating base (Figs. 6B, C). This area
 191 is characterized by an important content of cells in vacuole (Fig. 6G) and the evolution of these cells is oriented toward the
 192 epidermis. From the generating base to the tuber marrow, near the procambium, an analogous evolution of cells oriented
 193 toward the inner was observed (Fig. 6D). Cell development around this area was oriented on both sides of the generating

194 base represented by a line of generating cells. After that, medullary parenchyma composed by future conducting vessels
 195 (xylem and phloem) and amyloplast (Figs. 6B, E, F) was observed. At this stage, starch granules are not well observable.
 196 As the cuts approach the middle part, more precisely at the lower end of this part, the meristematic character of the
 197 generating base tends to disappear (Fig. 7). The parenchyma cells were more stained by the lugol with an intense
 198 coloration around the conducting vessels (Fig. 7B). Starch granules with different form arranged on the cell wall were
 199 observed (Fig. 7D, G). Toward upper end of the middle tuber part, conducting vessels differentiated, present a resorbed
 200 cytoplasm (Fig. 7E). The xylem with a striped, punctuated and thickened cell wall differed clearly from the phloem (Fig.
 201 7F). Cell wall thickening was observed on cortical parenchyma for a section realized at upper extremity of proximal part of
 202 the tuber (Fig. 8C). Some of them content starch granules stained orange yellow by the Marshall Red ammoniacal. The
 203 cells of the medullary parenchyma are more filled with starch grains whose size increases and variable form (Fig. 8E).
 204 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
 205 one) and 6.06 μm to 8.36 μm for the proximal part.
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 208 **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)
 209 Reconstitution of the cut (Gr X 40). (C) Cortical parenchyma (Gr X 100). (D) Generating base or procambium (Gr X 400). (E) Draft of

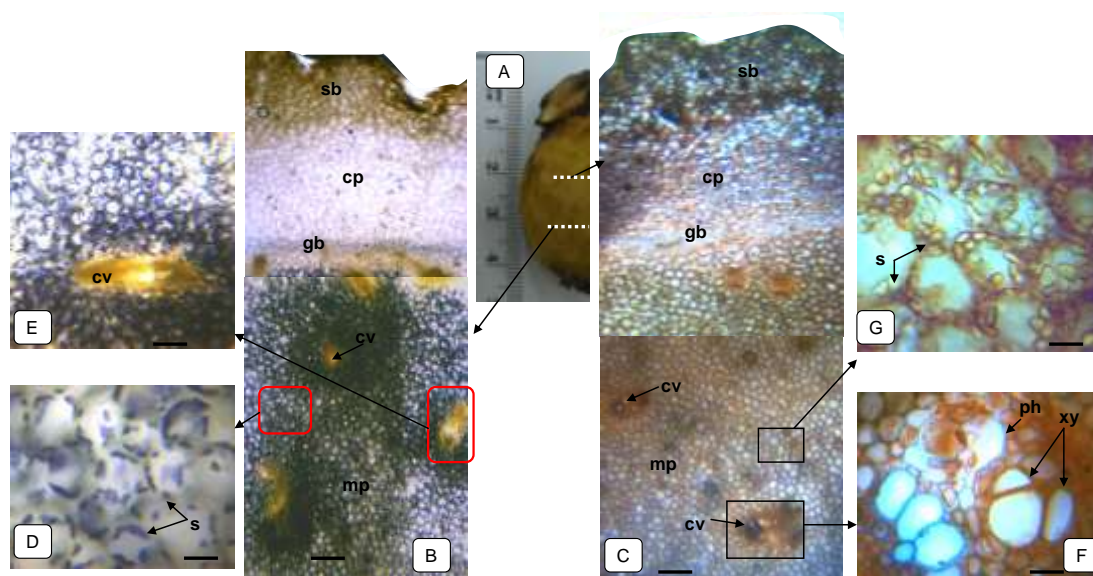
210 amyloplast stained by the lugol (Gr X 400). (F) Draft of conductive vessel with resorbed cytoplasm (rc) (Gr X 400). (I) Cortical
 211 parenchyma with colored substance (Gr X 400). From epidermis (ep) to the medullar parenchyma (mp), were observed the suberous
 212 area (sb) with five to six cells layers. Below, the cortical parenchyma (cp) made up cells with solid substance (ss) stained yellow by the
 213 lugol or not and vacuolated ones with meristematic activity near the generating base (gb). Below the procambium, the medullar
 214 parenchyma with undifferentiated conductive vessels (cv) and draft of amyloplast containing starch (s). Scale bars: 25 μ m.

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217 These starch grains observed in white light (Fig. 8F) have variable shapes (spherical, ellipsoidal and ovotriangular).
 218 Growth streaks are observable on certain grains. Certain conducting vessels observed in the distal and middle parts
 219 remain undifferentiated (Fig. 8D). It were presented as a stacking of bundle and not stained by the lugol reagent.

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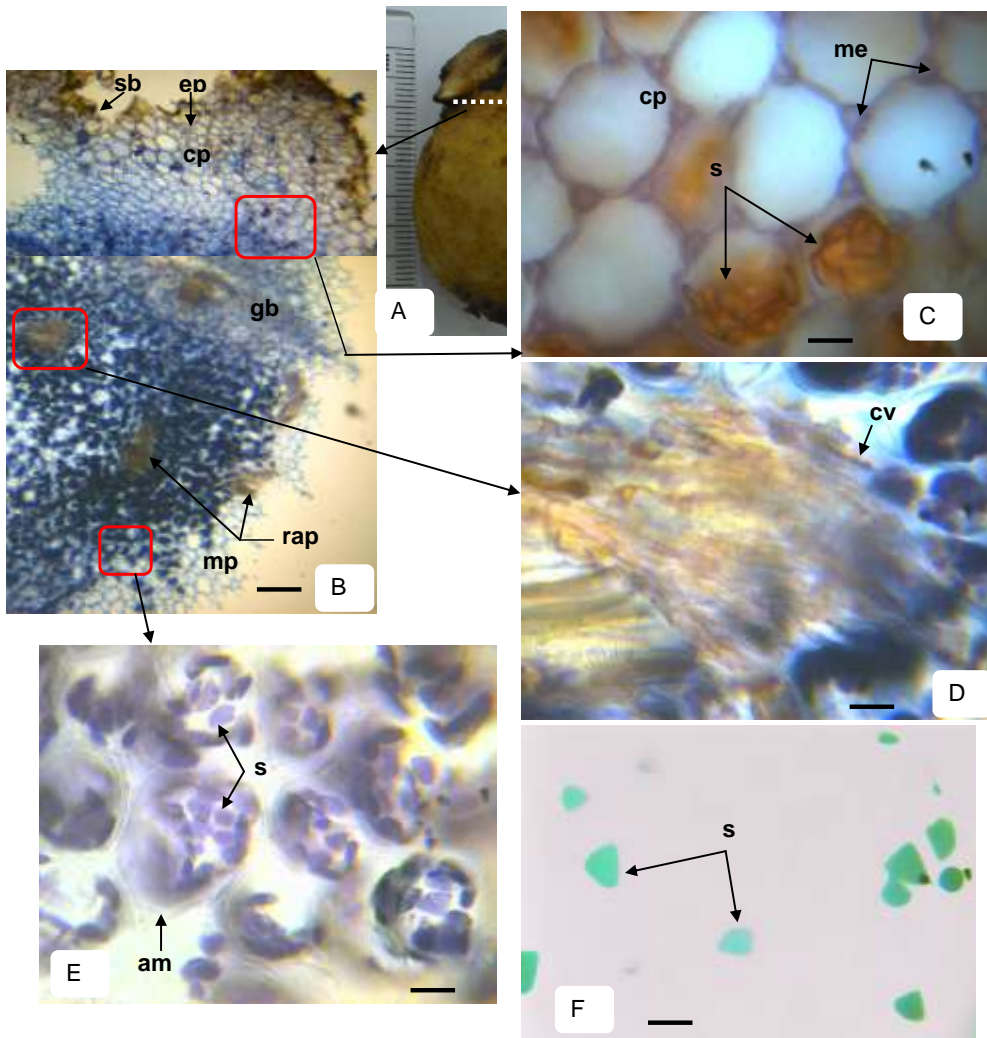
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222 **Fig. 7:** Cross section made at both ends of the middle part (var. kponan). (A) Tuber of 30 days of kponan variety. (B)
 223 Reconstituted cut of the lower end of the medial section (Gr X 40). (C) Reconstituted cut of the upper extremity of the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

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227 The tangential section (Fig. 9) reconstituted presents the two great parts separated by an annular generating cell layer as
 228 in cross section. The inner part to the cell layer is constituted of draft of conductive vessels and the outer part rich in
 229 calcium oxalate crystals. Lugol staining (Fig. 9B) shows no evidence of starch grains presence apart from the cambium
 230 cell layer which the vacuole is colored dark blue. These very vacuolated cells take a centrifugal orientation on either side
 231 of the generating base (Figs. 9D, H). The lower part of the procambium (towards the apex) is constituted of cells without
 232 particular orientation with irregular shape and size. It formed an aggregate of cutinized cells surmounted by a coif (Figs.
 233 9C, F, G). The cells at inner (medullar parenchyma) the section are arranged in line along the longitudinal axis (Fig. 9E).
 234 They are very close and vacuolated. Similarly, the conducting vessels are also arranged in line (Fig. 9I).

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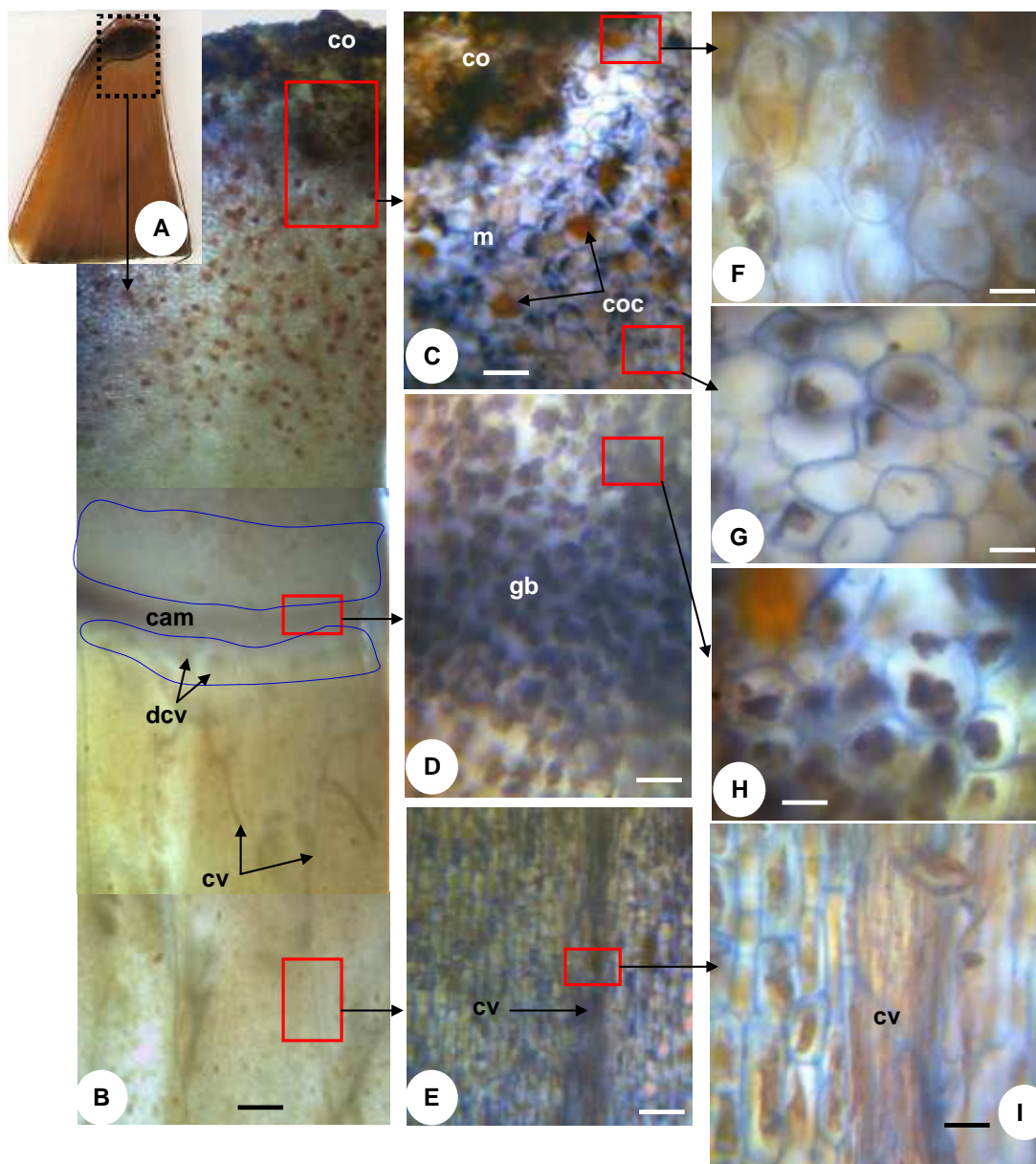
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Fig. 8: Cross section made at the upper end of the proximal part. (A) Tuber of 30 days of kponan variety. (B) Partial reconstitution of the cut (Gr : x 40). (C) Cortical parenchyma consisting of a few amyloplasts (Gr X 400). These thick-walled cells arrange space between them or meat (me). (D) Undifferentiated conducting 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.

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.



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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 µm.

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 (Figs. 9E, I).

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262 3.2. Scanning microscopy observation of yam tuber at thirty days after tuberization

263 Direct observations without metallization to gold powder by scanning electron microscope did not allow the yam tissue to
264 be observed at the beginning of tuberization. Samples of young yam tissues that are not rolled to gold powder tend to
265 deform during observation. Thus, a sample of three (3) months after the start of the Tuberization was selected. The three-
266 dimensional observation shows that the cells are generally of ovosphérique form with tapered end (Figs. 10A, B). They
267 are more like oval-shaped baskets suitable for filling by starch grains. This dimpled structure of irregular diameter also has
268 the allure of a honeycomb. The lower part of the distal area shows no traces of starch grains (Figs. 10A, B). But as the
269 sections approach the upper end, the first grains of starch appear around the conductive vessels (Fig. 10C). The filling of
270 the amyloplasts extends to the rest of the cells of the medullar parenchyma. This gives the appearance of a basket full of
271 grains (Fig. 10D). These starch grains generally have an oval triangular shape flattened in the longitudinal direction of the
272 triangle (Fig. 10F). The observed sample seems to show the only form. The conductive vessels at this stage of tuber
273 growth exhibit a completely resorbed cytoplasm giving the appearance of a pipe (Fig. 10E). The wall of these conductive
274 elements is perforated giving the appearance of a net (punctuation).

275 The beginning of yam tuberization results from a protuberance of the stem base due to the activity of the meristematic
276 cells, followed by the placement of the first tissues. The cambial activity ensures the growth in length and width of the
277 tuber (Fig. 11). The activity of the meristematic cells located at the apex actively participated in the development of the
278 tuber. The cambial activity of the generating base decreases from the distal to the proximal end. This activity responsible
279 for the longitudinal and radial growth of the tuber ceases at maturity of the tuber.

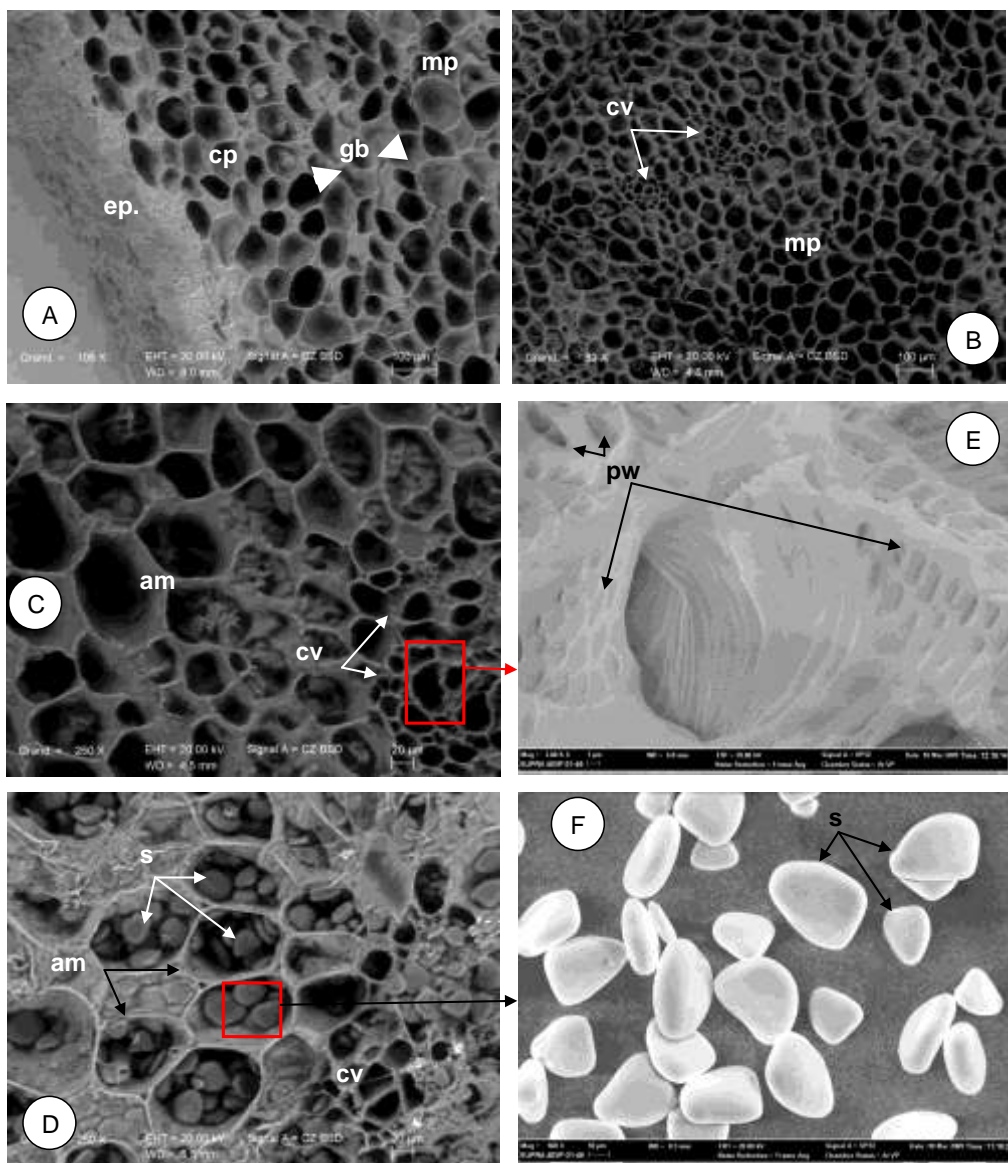
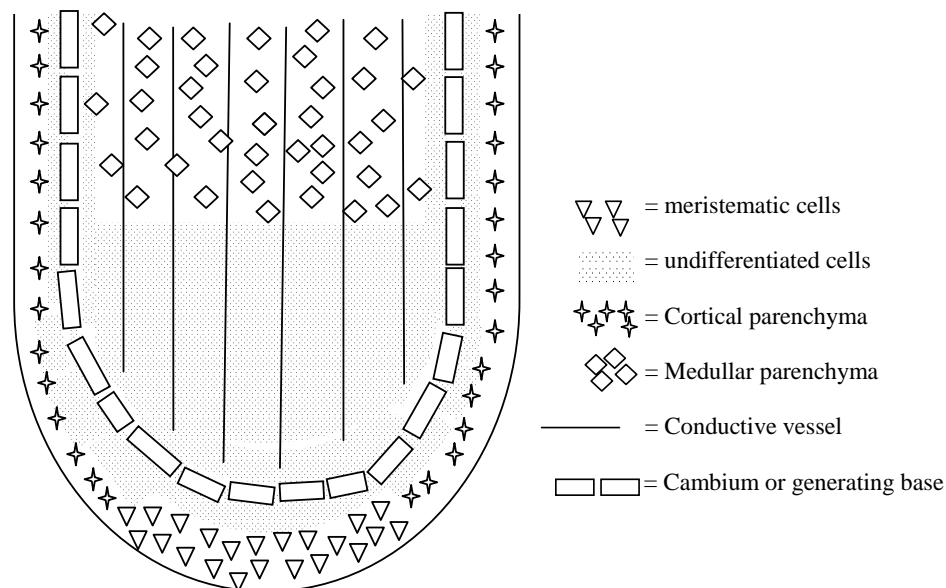


Fig. 10: SEM observation of a cross-section at the lower end of the middle portion of a sample of three months after the beginning of tuberization (kangba variety). (A) Cortical parenchyma. (B) Medullar parenchyma. (C) Conductive vessel. (D) Amyloplast. (E) Punctured wall of xylem. (F) Starch. am, amyloplast;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

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

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4. DISCUSSION

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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 (H. L. 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.

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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

317 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
318 wall. Indeed, in the case of tuber length growth, cell formation occurs at the level of the active zone observed on either
319 side of the cambium in the longitudinal plane. Once formed at the apical meristem, these cells differ a little more towards
320 the medial part of the tuber and constitute the different tissues of the yam tuber. The sense of this differentiation is
321 centripetal. This phenomenon is responsible for the elongation of the tuber. This growth gradient was revealed by the
322 study of the morphogenesis of Tuberization performed by Trouslot [9]. But as the tuber approaches maturity, growth slows
323 and ends in a month of senescence always according to the same source. This results in a decrease in the proportion of
324 undifferentiated tissues. The meristem activity of the cambium would then be reduced. For thickness growth of yam tuber,
325 this could still be a "cambiale" activity of the generating base that would be the origin. In fact, cross sections showed that
326 on both sides of the generating base, a layer of highly vacuolate cells emanating directly from it was observed. This
327 production of cells whose differentiation would give the cortical parenchyma on the outer side of the generating base and
328 the medullary parenchyma on the inner side would cause growth in tuber thickness. This particular type of cambial tissue
329 exists in some Liliaceae and Dioscoraceae [13]. According to Lawton and Lawton [15], in elongated tuber species, a
330 highly meristematic apex develops; the activity at a certain distance from the summit would cease. In spherical tuber
331 species, the meristem would remain active on the entire surface of the tuber until maturity. This was observed in this
332 study.

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334 **4. CONCLUSION**

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

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