

## DEVELOPMENTAL MORPHOLOGY OF SEEDLINGS OF *DIOSCOREA CAYENENSIS* - *D. ROTUNDATA* COMPLEX<sup>1</sup>

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### Abstract

The developmental morphology from the embryo to two-month-old yam (*Dioscorea cayenensis* - *D. rotundata* complex) seedlings was studied by light microscopy. During the first month after seed sowing, the organs which closely tied in with the basal axial part of the young seedling plant initiated and/or developed; these included the cotyledon, the first three leaves, the root system and the tuber. The first leaf, present in the embryo, was peculiar because it lacked the axillary bud. The second leaf turned into a scale and subtended the developing axillary system. The internode underlying the third leaf became the first elongated shoot. Adventitious roots arose from the basal axial part. As soon as the 15th day after sowing, the tuber formation was initiated by anticlinal and periclinal divisions in the parenchyma of the primary root-cotyledon axial region enclosed at this stage within the cotyledonary sheath. Two months after sowing, the very young tuber became organized, with a large meristematic apex covering parenchyma and provascular strands.

**Key Words :** *Dioscorea cayenensis* - *D. rotundata* complex developmental morphology, seedlings.

Yam is a staple food crop in West Africa within the yam zone (Miège 1954, Coursey 1965, 1967), as also in the Caribbean, a part of South-East Asia and the Pacific islands. Many cultivars of the African *Dioscorea cayenensis* - *D. rotundata* complex are most-widely cultivated by vegetative propagation, along with those of the Asiatic *D. alata* (Coursey 1967, 1982). Hybridization and propagation through seeds are new attempts to improve the yam (Sadik & Okereke 1975a, Sadik & Rockwood 1975, Degras 1982, Touré & Ahoussou 1982).

Although the morphology of the young seedling, the anatomy, origin and development of the tuber in various Dioscoreaceae seedlings have been reported (Queva 1894, Sparshott

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1935, Martin & Ortiz 1963, Lawton & Lawton 1967, 1969, Sharma 1974, 1976, Sadik & Okereke 1975b), the development of the tuber in seedlings of these two major yam crops has never been investigated. In the present paper the developmental morphology of young seedlings of *D. cayenensis* - *D. rotundata* is described.

### Material and Methods

Fruits of the cultivar Sépélo (*Dioscorea cayenensis* Lamk. - *D. rotundata* Poir. complex) were collected from farmer's field at Ano Simbé, Côte-d'Ivoire. Seeds were germinated in petri dishes on moist filter paper and the seedlings replanted first in vermiculite and then in compost. They were grown in a growth chamber with a 12h:12h light:dark cycle at 25°C.

At irregular intervals after sowing, seedling samples were fixed in FAA (formaldehyde-acetic acid-ethanol), dehydrated and embedded in paraffin, sectioned at 7  $\mu\text{m}$  on a rotary microtome and stained with Regaud's hematoxylin. Seeds were soaked in water before fixation of the embryo.

### Observations

EMBRYO — The cotyledon part of the embryo consisted of a fan-shaped lamina, a petiole and a sheath of the cotyledon (Fig. 1A-C). The plantlet proper part of the embryo consisted of a root (Fig. 1A,D), and the shoot apex itself was composed of an apical meristem and the first leaf (Fig. 1B,C,E). The root and the shoot apices were not in the same plane. The cotyledonary sheath completely surrounded the plantlet proper part (Fig. 1A-D); a slit gave access to the cavity containing the apical meristem and the first leaf (Fig. 1E).

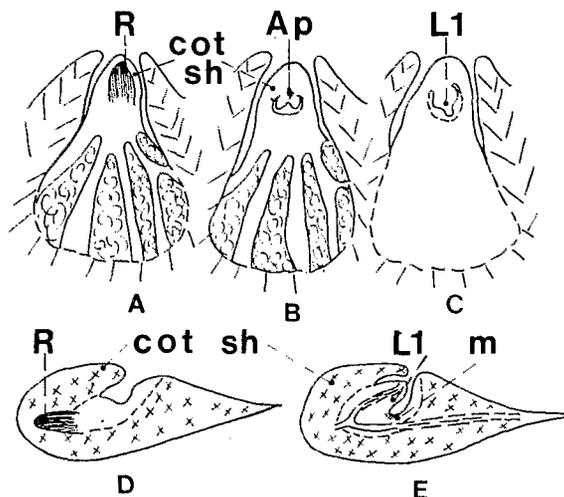
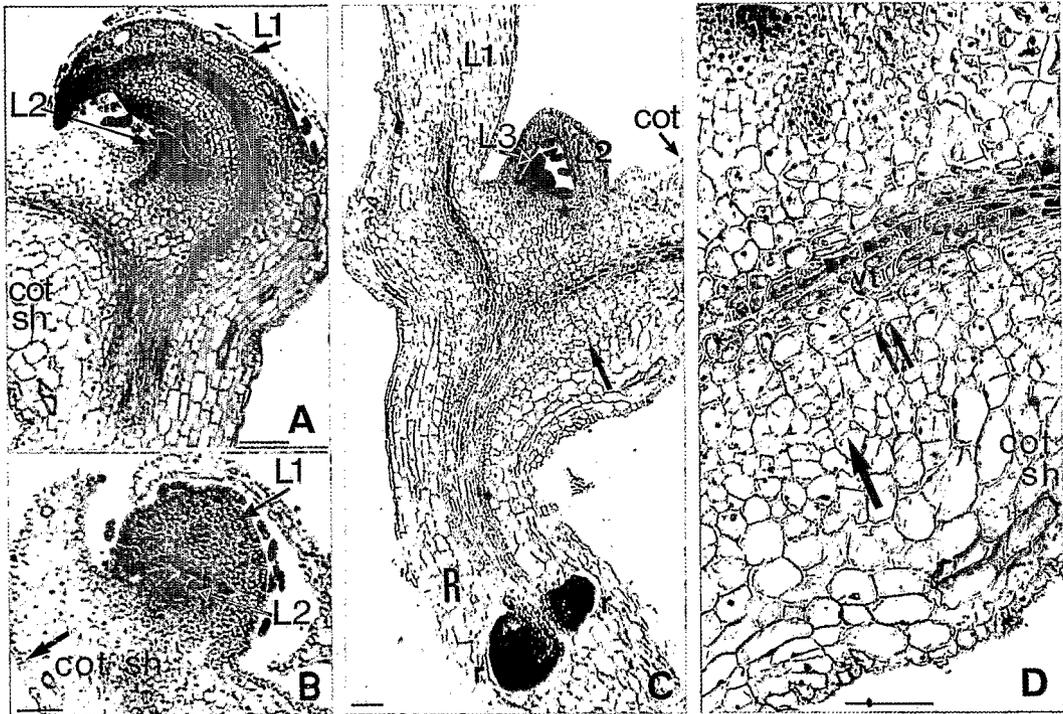


Fig. 1A-E — Sections of the embryo within the seed. (*Ap*, shoot apex; *cot sh*, cotyledonary sheath; *m*, shoot apical meristem; *L1*, first leaf; *R*, primary root). Camera lucida longitudinal sections parallel (A-C) and perpendicular (D,E) to the flat plane of the cotyledon.

**SEEDLINGS** — Eight to ten days after sowing, the root and opposite to it the first leaf protruded out of the cotyledonary sheath; the second leaf primordium emerged (Fig. 2A). The cotyledonary sheath had a much wider surface area (three to four times as much) in comparison with the apex; it also exhibited small darkly-stained cells in the epidermis and large vacuolated ones in the parenchyma (Fig. 2B).

At day 15 after sowing, rootlet primordia formed from the primary root (Fig. 2C); at this stage, the primary root was frequently the only root developed, though in some samples young primordia of adventitious roots arose below the shoot apex. The basal part of the first leaf petiole enlarged and was given off opposite to the cotyledon. The young second leaf overlapped the meristem which appeared in its axil, the third leaf primordium and the shoot apical meristem (Fig. 2C). At this stage, some anticlinal and periclinal divisions appeared in the parenchyma of the primary root-cotyledon axial region enclosed within the cotyledonary sheath: they represented the first stage of tuber initiation (Fig. 2C,D). This zone of divisions extended towards the vascular tissue and was always situated on the side next to the cotyledon, at the levels where the vascular tissues of the cotyledon and of the first leaf converged on the central cylinder of the primary root.



**Fig. 2A-D** — (*cot*, cotyledon; *cot sh*, cotyledonary sheath; *L*, leaf; *R*, primary root; *r*, rootlet; *vt*, vascular tissue). **A,B.** Sections of 10-day old seedlings. **A.** Median longitudinal section showing the first leaf, the second leaf primordium *L2* and the wide cotyledonary sheath. **B.** Cross section through the cotyledonary sheath; note epidermal cells (arrow). **C,D.** Median longitudinal section of a 15-day old seedling. **C.** The second leaf surrounds the third leaf *L3* and exhibits an axillary meristem (star). **D.** Same enlarged, showing anticlinal (arrow) and periclinal (double arrow) divisions towards vascular tissue between the primary root and the cotyledon. Bar=100  $\mu$ m.

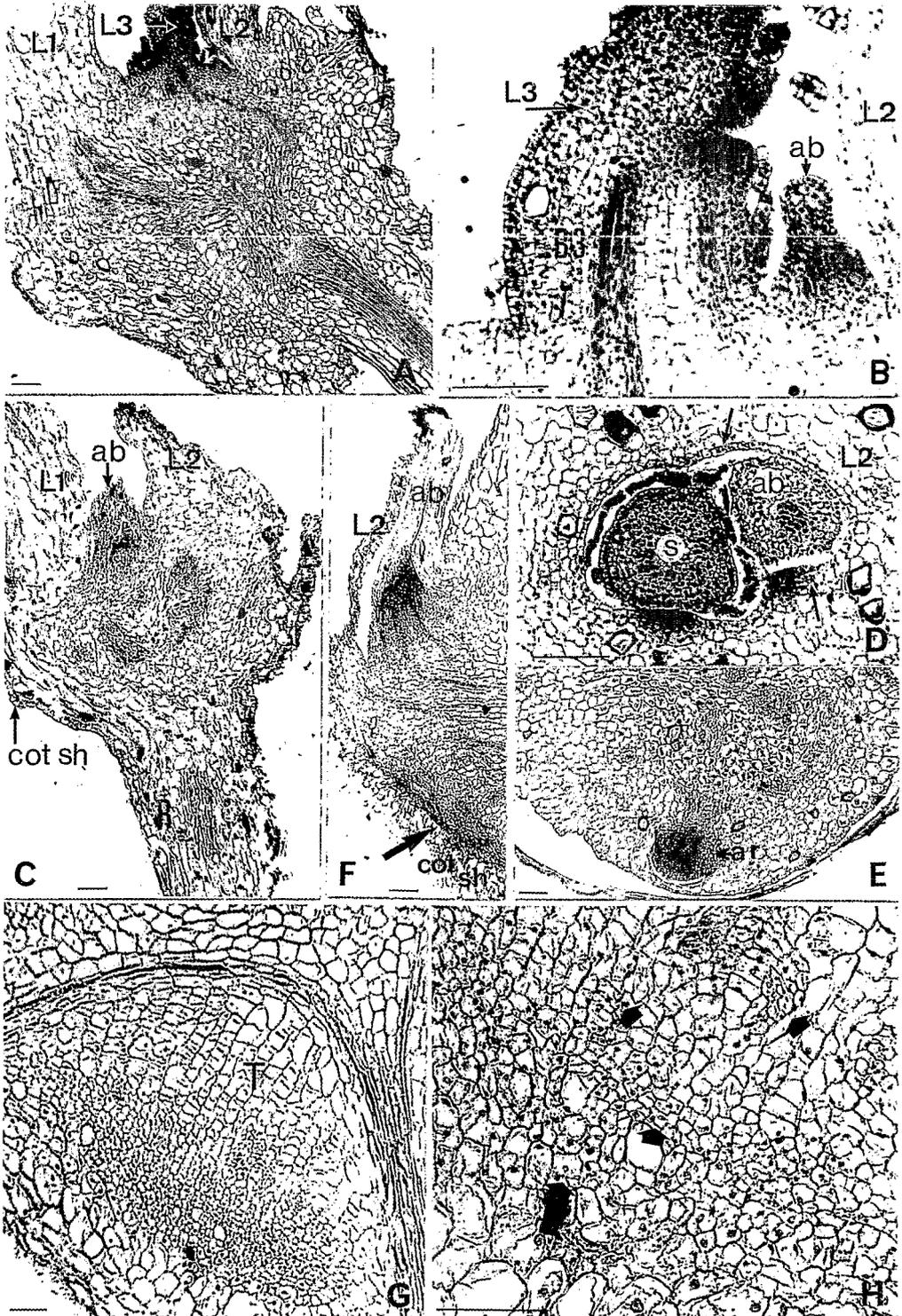


Fig. 3A-H

At day 25 after sowing, the first leaf had expanded and was green. The second leaf, with slightly stained vacuolated cells of parenchyma, turned into a small sheathing scale without its petiole differentiating (Fig. 3A,D). The third leaf and its subjacent basement both grew together, such that the internode between the second and the third leaf became the first elongated shoot (Fig. 3B). Sections revealed important events concerning the axillary system of the scale (second) leaf: the axillary bud had two leaf primordia (Fig. 3C) and superficial accessory meristems appeared out-of-axil on the adaxial surface of the scale (Fig. 3D). Moreover, adventitious roots arose between the primary root and the first elongated internode of the stem, mostly from the axial zone below the scale (second) leaf with its axillary bud (Fig. 3E).

Two months after sowing, the seedlings had four expanded leaves. The tuber was weakly- or non-protruding. When non-protuberant, it remained enclosed within the cotyledonary sheath on the side just next to, and below the axillary bud of, the scale (second) leaf (Fig. 3F). In the median region of the very young tuber, the apex of the tuber was the large darkly-stained zone, 0.8 mm x 0.1-0.2 mm, showing cells which divided in various directions (Fig. 3G, H). This apex covered rows of parenchyma cells and provascular strands; it was still surrounded by the large remaining cells of the cotyledonary sheath (Figs 3F-H, 4A). At this stage, the axillary bud of the scale (second) leaf branched, its first-formed leaf subtending a new meristem with a leaf primordium (Fig. 4B).

At a later stage of seedling development, a meristematic subapical zone, located beneath 10-15 peripheral cell layers, became discernible at the apex of the structured tuber (Fig. 4C,D).

### Discussion

The present investigation reveals that in *Dioscorea cayenensis* - *D. rotundata* complex, meristematic activity during the first month after seed sowing contributed to the initiation and/or to the growth of organs closely tied in with the basal axial part of the young seedling. These organs include the cotyledon, the first leaf, the second leaf with its axillary system, the third leaf with its basement, the root system and the tuber itself.

Our observations on *D. cayenensis* - *D. rotundata* complex point out that the first leaf, present in the embryo, was green in colour when it expanded and was peculiar because it lacked the axillary bud. The presence of first leaf in the embryo has been reported

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Fig. 3A-H — (ab, axillary bud; ar, adventitious root; B, basement; cot sh, cotyledonary sheath; L, leaf; R, primary root; s, shoot; T, tuber). A-E. Sections of 25-day old seedling plants. A,B. Longitudinal sections. Note the third leaf above its elongated subjacent basement and the scale (second) leaf with its axillary bud. C. Axillary bud with leaf primordia in the scale (second) leaf axil. D. Cross section through the shoot and the axillary bud of the scale (second) leaf. Note the out-of-axil superficial meristems (arrow). E. Cross section through the zone of adventitious roots below the axillary bud of the scale (second) leaf. F-H. Longitudinal sections of 2-month old seedling plants. F. Location of the apex of the tuber (arrow). G. Median section of the very young tuber. H. Enlargement showing various divisions (arrows) in the apex of the tuber. Bar=100  $\mu$ m.

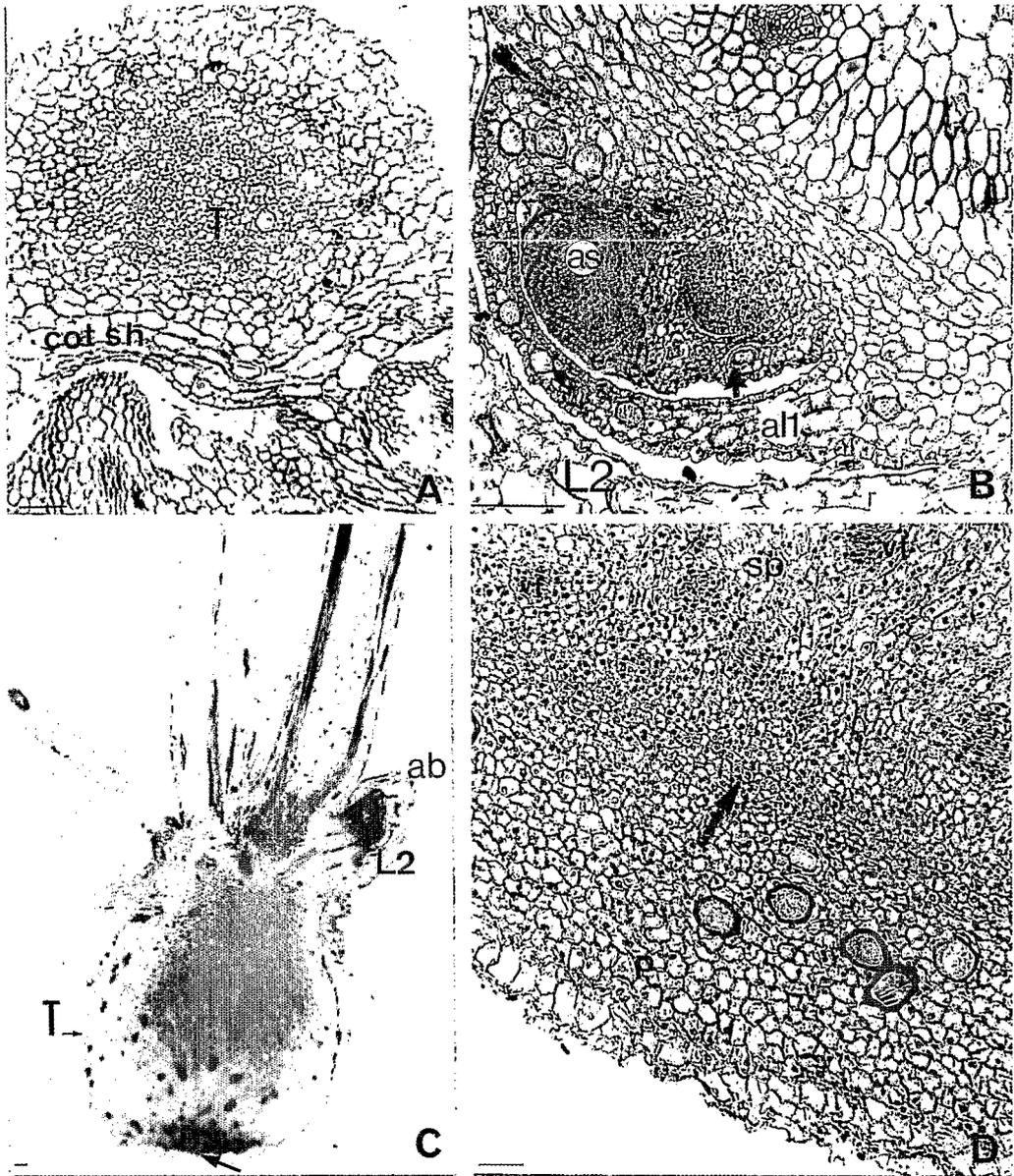


Fig. 4A-D — (*ab*, axillary bud; *al*, leaf of axillary bud; *as*, shoot from axillary bud; *cot sh*, cotyledonary sheath; *L*, leaf; *p*, parenchyma; *sp*, starch-storing parenchyma; *T*, tuber; *vt*, vascular tissue). A,B. Cross sections of 2-month old seedling plants. A. Through the very young tuber surrounded by the cotyledonary sheath. B. Through the branched axillary bud of the scale (second) leaf. The first leaf of the bud around the young shoot has its own axillary bud (arrow). C,D. Longitudinal sections of 3-month old seedling plants. C. The well structured tuber, the apex of which is shown by the arrow; note the scale (second) leaf and its axillary bud. D. Enlargement of the apex of the tuber. Note the subapical meristematic zone (arrow). Bar=100  $\mu$ m.

in *D. rotundata* (Sadik & Okereke 1975b) and in other species of Dioscoreaceae (Beccari 1870, Bucherer 1889, Queva 1894, Gardiner & Hill 1902, Smith 1916, Nagaraja Rao 1953, Lawton & Lawton 1967). According to Sharma (1976), a bud may as an exception arise in the axil in *D. deltoidea*.

Results of this study highlight the great extent of the axillary system of the scale (second) leaf. The sequence of events, that lead to the formation of several buds in the scale leaf axil of the seedlings of *D. cayenensis* - *D. rotundata* complex, included : axillary meristem initiation (15th day), axillary bud formation and accessory meristems initiation (25th day), and branching of the axillary bud (2nd month). These buds of the axillary system of the scale have the potential to develop into basal lateral shoots. The scale (second) leaf subtending its axillary bud has been generally observed in young seedlings of other species of Dioscoreaceae (Queva 1894, Goebel 1905, Sparshott 1935, Martin & Ortiz 1963, Lawton & Lawton 1969, Sharma 1974, 1976). Moreover many Dioscoreaceae members produce a series of buds in the leaf axils (Queva 1894, Dale 1901, Burkill 1960). Shah et al. (1969) have reported differentiation of two accessory bud meristems on the side of the axillary bud in *D. alata*, while in *D. sansibarensis* two to four accessory buds were reported to be derived from leaf axils (Rao & Tan 1976). In *D. cayenensis* - *D. rotundata* complex reported here, accessory meristems arose from the epidermis or the subepidermis on the adaxial surface of the scale in an out-of-axil position. Epidermal origin of adventitious bud on the hypocotyl of *Linaria triphylla* has been reported (Champagnat 1961) as well as subepidermal origin of hypocotyledonary buds in *Euphorbia peplus* (Champagnat et al. 1962). The growth of the third leaf marked the beginning of shoot elongation.

The formation of adventitious roots, which play a major part in the root system of the seedling, occurred at the same time as the tuber initiation between 15 and 25 days after seed sowing. Adventitious roots arose from basal axial part of the seedling which formed from the basements of the cotyledon, the first leaf and the scale (second) leaf with its axillary bud. Histological sections in this study show anticlinal and periclinal divisions in the parenchyma of the primary root-cotyledon axial region, on the side next to the cotyledon, as the earliest evidence of tuber initiation. A parallelism in sites of origin of the seedling tuber (root-cotyledon axis) and root system (root-scale [second] leaf axis) may be thus established. Later the very young tuber became organized while it was still enclosed within the cotyledon sheath on the side next to, and below the axillary bud of, the scale (second) leaf. Its meristematic apex capped parenchyma cells and provascular strands both of which appear to be differentiated from cells arising from the apex. This is the first presentation of tuber formation in seedlings of *D. cayenensis* - *D. rotundata* complex. This mode of tuber formation is in agreement with some conclusions made for other Dioscoreaceae members by Queva (1894) and Martin & Ortiz (1963). The annual tuber was sometimes formed by a "point de végétation" localized in the hypocotyledonary hypertrophy which appeared as the result of divisions in various directions (Queva 1894). In two American species of *Dioscorea*, the perennial tuber arose from a bulge which resulted from rapid cell division of the ground parenchyma of hypocotyl. Its tissues arose directly from the ground meristem; its further growth might be considered as resulting from the activity of a cambium-like layer which appeared as a direct continuation of the primary thickening meristem near the apex of the tuber (Martin & Ortiz 1963). According to Lawton & Lawton (1969), in five species of

*Dioscorea*, the first indication of tuberization was the formation of a perivascular cambium in the hypocotyl region, and the shape of the tuber depended on the local persistence of meristematic zone. According to Sharma (1974, 1975), in *D. glabra*, the tuber originated in the primary thickening meristem initiated in the perivascular region of the hypocotyl, tuber elongation being due to the activity of a deep-seated apical region of this meristem.

The present study shows that the foliar basements of the cotyledon, of the first and second leaf enlarged but did not elongate. At first, the axillary system of the scale was closely connected with the zone of adventitious roots above the tuber; that could explain the great vigour of the basal lateral shoots observed when seedling plants have been cultivated under tropical field conditions (Trouslot 1985). Later, in the process of development the basal axial part of the seedling became composed of telescoped internodes and nodes, and bore the cotyledon, the first leaf, the scale (second) leaf with its axillary system, the shoot, the primary root, adventitious roots and the tuber. By maturity, this basal axial part had developed to become the head of the tuber, always connected with the tuber itself and with the axillary system of the scale. A homology between the basal axial part of the seedling and the pretuber (Miège 1952) of vegetatively propagated yams is thus established.

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