# External and Internal Morphology of Seed Germination and Seedling Establishment in the White Guinea Yam, *Dioscorea rotundata* Poir

**Okezie, C. E. A., Okonkwo, S. N. C. and Nwoke, F. I. O.**
Department of Botany, University of Nigeria, Nsukka

**Corresponding author:** Okezie, C. E. A. Department of Botany, University of Nigeria, Nsukka, Nigeria

**Abstract**

***The external and internal morphology of the process of seed germination and seedling establishment in "Obiaoturugo" variety of the white Guinea yam, Dioscorea rotundata Poir is hereby reported. Anatomical investigation of the seed embryo was carried out at different stages during germination in order to relate the observed morphological changes to the cognate physiological and biochemical events driving the germination process. The presence of only one fan-shaped cotyledon highly traversed by vascular strands which serve for the conduction of nutrients from the surrounding endosperm, and at least two pairs of leaf primordia, have been clearly elucidated. It is proposed that differential cell division among the tissues of the shoot apex, in which one of the leaf primordia elongated faster at the expense of the others, accounts for the alternate leaf arrangement on the vine of the ensuing seedling from seed germination in this variety of D. rotundata.***

**Keywords:** External and internal morphology, Seed germination, *Dioscorea rotundata*

## Introduction

Over the years, the progagules normally employed for yam (*Dioscorea* spp.) propagation are seed yams, which are whole tubers specially cultivated for that purpose. Yams are also propagated with setts which are cut-up ware yams, and, more recently, with minisetts (Okoli *et al.*, 1982; Ndon and Ndaeyo, 2002) which are even smaller (usually less than 30 grammes) than setts. The larger the planted material, the greater is the yield (Miege, 1957; Nwoke *et al.*, 1973). Thus the tuber serves both for food and for perennation. It has been estimated that up to 30% of farmers' crops must be used to plant in the following year (IITA, 1999), thus contributing to its (food) scarcity for a better part of the year. Coupled to this is the great amount of labour (as in land clearing, weeding, staking, single or double harvesting, and barn preparation) required to produce yam (Onwueme, 1978).

Problems associated with yam production, as enumerated above, have resulted in the search for alternative and more economical modes of propagation. Of all the known alternatives, propagation by seed is the only one by which the crop could be improved through conventional breeding. The sexual reproductive process which produces the seed also results in hybridization. This in turn results in the production of new genotypes which can form the basis for selection (Onwueme, 1978). A population improvement scheme has since been initiated to generate seed populations of *Dioscorea rotundata* containing high frequencies of desirable characteristics (Wilson, 1978a and b).

With the successful establishment of yam populations through seed germination (Waitt, 1963; Sadik and Okereke, 1975) efforts have been made to understand the physiology of the germination process and the proper establishment of the taxonomic status of *D. rotundata*. Most such previous studies were based on the external morphological features of the embryo and the associated emergent structures. The present study aims, for the first time, to carry out detailed anatomical investigation of the seed embryo of "Obiaoturugo" variety of *D. rotundata* at different stages during germination, with a view to relating the observed external and internal morphological changes to the cognate physiological and biochemical events driving the germination process.

## Materials and Methods

**Source of seeds:** The seeds used in this study were harvested in December, 2005 from fruits of flowering populations of "Obiaoturugo" variety of the white Guinea yam, *D. rotundata* Poir. The trilocular fruits were sun-dried for 96 hours during which longitudinal cracks developed at the median ridge of each locule, thus enabling easy manual seed extraction.

**Seed extraction and storage:** Seeds were extracted by application of gentle vertical pressure on the dry fruit, thus allowing the winged seeds to drop on a flat dry receptacle. Filled seeds were then selected and manually dewinged, sun-dried for a further 48 hours before being subjected to post-harvest storage for at

least 12 weeks, in order to ensure that their dormancy was broken prior to use for germination studies. The dry seeds were packaged in dry brown envelopes at 50 seeds per pack and stored on a wire mesh over dry calcium chloride crystals in a desiccator and withdrawn as desired for this study.

**Seed germination studies:** Non-dormant filled seeds were surface-sterilized in 1% sodium hypochlorite, rinsed in four changes of sterile distilled water, and placed on moist 9cm – diameter Whatman No. 1 filter paper in Petri dishes and allowed to germinate in a dark incubator (Gallenkamp, Made in England) at 27 ± $2^0$C. The seeds were sampled daily for the external and internal morphological features in course of germination.

Prior to sampling, the embryos were carefully dissected out of the enclosing structure (endosperm and testa) under a binocular microscope (Karlkaps Assler/Wetzlar, Made in Germany) and their dimensions measured by means of a calibrated ocular micrometer (Erma Optical Works Limited, Japan).

During sampling, embryos were fixed in FAA and later dehydrated in a graded alcohol series. During dehydration, they were stained with eosin in order to make handling in wax easy. They were then infiltrated with $30^0$C melting point paraffin wax and later embedded in $56^0$C melting point wax by the method of Sass (1958). Serial longitudinal sections of the embryos were cut 10µ thick by means of a rotary microtome ("820" Spencer Microtome supplied by American Optical Corporation). The serial sections were fixed on clean microscope slides by means of freshly prepared egg albumen (an adhesive) over a warm plate for 24 hours. They were then stained in aqueous safranin for one hour and passed through 30, 50, and 90% ethyl alcohol and counterstained in fast green in 95% alcohol for 10 seconds. The sections were cleared in carbol-xylene, and then mounted by covering with Canada balsam and cover slips and examined under the microscope.

## Results

The typical *D. rotundata* (var. Obiaoturugo) embryo is a fan-like structure which lies in a cavity between the two halves of the seed endosperm. The non-dormant embryo is always longer than wide, measuring 900-1100µ in length and 750-800µ in breadth when dissected out from the seed after soaking in water for 24 hours. The wider fan-like portion is the cotyledon traversed by groups of vascular strands (Plate 1). The embryo tapers towards the narrow root axis. This region is globular and consists of a cavity within which lies a dark oval structure which would later differentiate into the root primordia towards the narrow end and the shoot primordia towards the wider fan-like region. The cells of the cotyledon are large and parenchymatous, and decrease in size towards the root axis of the embryo (Plate 1).

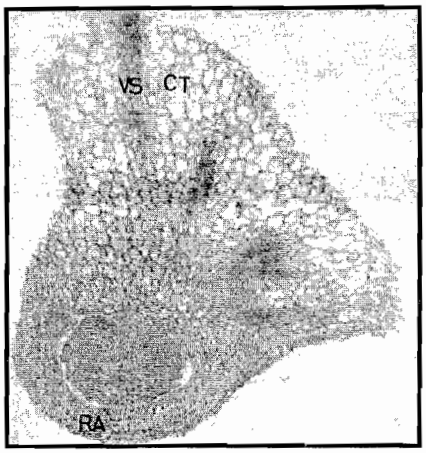



**Plate 1: A longitudinal section through the flat axis of a mature *D. rotundata* embryo after a 24 hour moisture imbibition. Note the root axis (RA) at the narrow end and the fan-shaped cotyledon (CT) traversed by vascular strands (VS), x 400.**

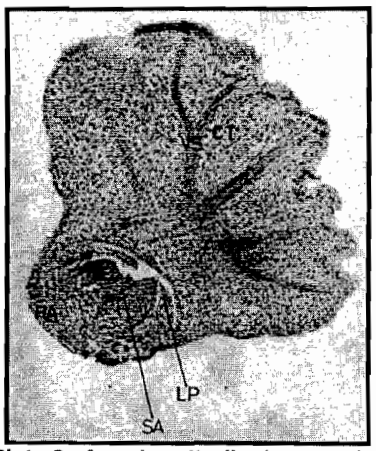
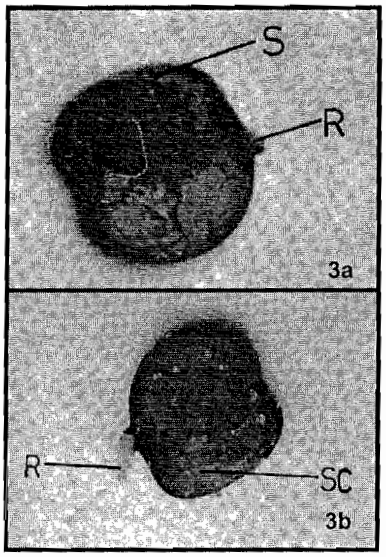
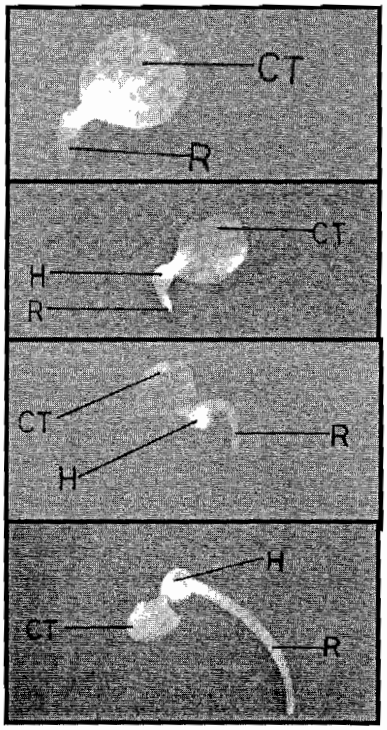


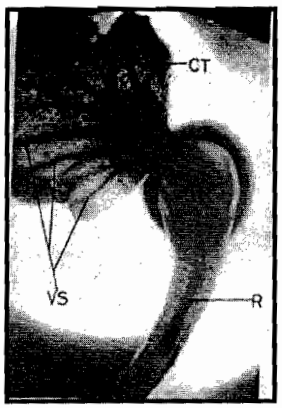

**Plate 2: A longitudinal section through the flat axis of *D. rotundata* embryo on the 7th day of moisture imbibition. Note one of leaf primordia (LP) that flank the shoot apex (SA) while the root apex (RA) is in the opposite direction to the shoot apex (SA). Note also the linked, now more prominent vascular strands (VS) traversing the fan-shaped cotyledon (CT), x 400.**




Plates 3a and 3b: *D. rotundata* seed (S) at the earliest visible sign of germination: when the radicle (R) had barely emerged from the concave end of the seed (S) (3a) and when the radicle (R) had elongated and become very obvious after rupturing the seed coat (SC), x 25.




Plates 4a - 4d: The germinating *D. rotundata* embryo (a-d) dissected out of the seed, showing the cotyledon (CT), the radicle (R) and the hypocotyl (H) bulging as the radicle grows longer (4d), x 20.




Plates 5: A close-up of germinating *D. rotundata* embryo with an enlarged hypocotyl (H) and prominent vascular strands (VS) which conduct nutrients from the seed endosperm through the cotyledon (CT) to the growing radicle (R), x 400.

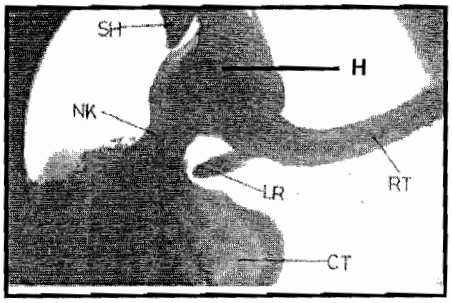



Plate 6: A close-up of *D. rotundata* seedling on the 3rd week during germination, when the shoot (SH) has emerged from the expanded hypocotyl (H) and growing in the opposite direction from the main root (RT) and the newly formed lateral root (LR). The root and shoot form a continuous axis lying at right angles to the absorptive cotyledon (CT) from which nutrients flow through a neck-like structure (NK) to the growing root and shoot, x 400.

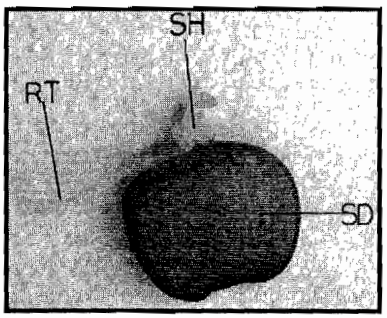



Plate 7: The external features of a young *D. rotundata* seedling with a well developed first root (RT) and a newly emerging shoot (SH) from the seed (SD), x 25.

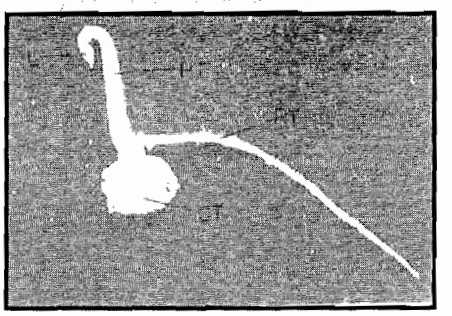



**Plate 8: A young *D. rotundata* seedling dissected out from the seed, showing a fully elongated first hook-like photosynthetic leaf with a prominent petiole (PT), a yet-to-expand leaf blade (L) and a prominent primary root (RT) all still attached to the cotyledon (CT), x 20.**

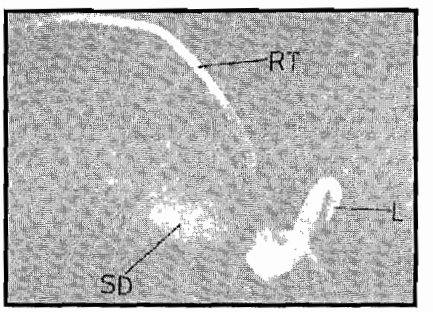



**Plate 9: The external features of a young emergent *D. rotundata* seedling on the 4th week of germination, with a second root developing in addition to the fully developed primary root (RT). The first photosynthetic leaf (L) has become prominent and hook-like while the cotyledon is still within the seed (SD), x 40.**

A longitudinal section through the flat surface of the embryo by the 7th day of moisture imbibition reveals that the shoot apex is flanked by pairs of leaf primordia which have begun to differentiate into young primordial leaves, while within the fan-like single cotyledon, the vascular strands have become prominent and all appear to be linked (Plate 2). The cells of the embryo are also distinctly nucleated at this time.

The first externally visible sign of seed germination is the protrusion of a radicle (an outgrowth of a root primordium) from the concave end of the seed (Plate 3a) as a result of the elongation of cells of the root apex of the embryo, thereby rupturing the seed coat (Plate 3b) as this extension growth continues. This radicle protrusion occurs between the 7th and the 14th day from moisture imbibition by the seed.

Once the radicle has burst through the seed coat, it tends to curve as it grows longer (Plates 4a, b and c). The hypocotyl (the junction between the root and shoot axis) exists as a prominent bulge which continues to enlarge as the radicle elongates (Plate 4d).

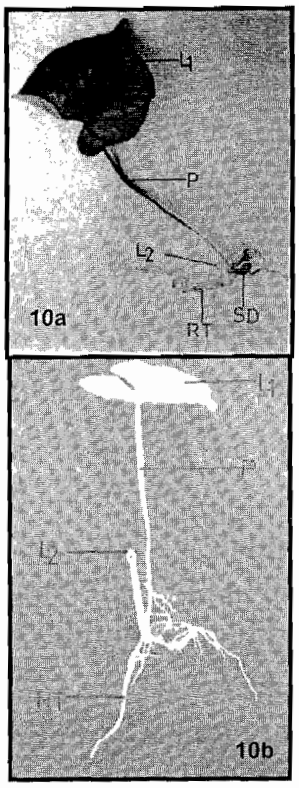
With increased mobilization of nutrients from the endosperm through the prominent vascular strands (Plate 5) to the now prominent hypocotyl by the 3rd week from imbibition, the shoot emerges from the expanded region of the hypocotyl and grows in the opposite direction from the root axis (Plate 6). The root and shoot, growing in different directions, form a continuous axis lying at right angles to the absorptive cotyledon. There is a prominent junction (the neck) between the root/shoot axis and the cotyledon through which nutrients absorbed from the endosperm by the cotyledon pass to the growing seedling (Plate 6). The external features of the seedling at this time reveal that it consists of a prominent root and shoot joined together at the region of the hypocotyl (Plate 7).

The first photosynthetic leaf consists of a prominent petiole which terminates in a hook-like blade (Plate 8). Plate 9 shows the external features of the entire seedling, consisting of the root/shoot axis still attached to the seed on whose endosperm the young seedling depends for nourishment for further growth. After the first leaf has expanded into a full blade, the second leaf takes off from the base of its petiole (Plates 10a and b). Subsequent leaves develop one at a time (alternately) each from the base of the petiole of the preceding leaf (Plates 11a and b), and thus a full seedling is established within 4 weeks from commencement of germination.

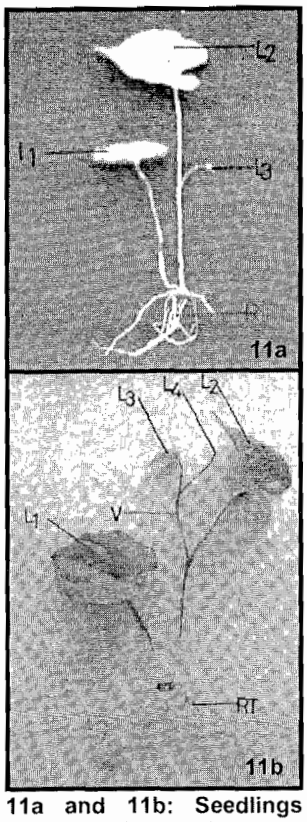
## Discussion

Earlier studies on seed germination in *D. rotundata* (Waitt, 1963; Sadik and Okereke, 1975) focused mainly on the external features leading up to seedling establishment without correlating these with the physiological processes that ensure germination. In our present study, detailed anatomical studies of the embryo have been employed in order to relate the internal morphological changes and cognate physiological and biochemical events that drive the germination process leading to the observed morphological changes of the seedling at the various stages of germination in this species.

As the mature viable seed imbibes water during the first 48 hours in a moist, well aerated environment and at ambient temperature, the single cotyledon of the embryo expands and fills the slit between the two halves of the endosperm. Contrary to the observations on other *Dioscorea,* namely; *D. opposita* (Rao, 1953) and *D. bulbifera* (Lawton and Lawton, 1967) embryos, neither a first leaf nor a plumular bud were noticed in the embryos of *D. rotundata* employed in our study, at this stage (i.e. 48 hours from water imbibition).




**Plates 10a and 10b: *D. rotundata* seedlings showing a fully expanded first photosynthetic leaf ($L_1$) with a prominent petiole (P), the second leaf ($L_2$) (10a) originating from the base of the first leaf which later elongates (10b), a number of roots (RT) and the remains of the seed (SD), x 1.5.**




**Plates 11a and 11b: Seedlings of *D. rotundata* showing the sequential production of leaves ($L_1$, $L_2$, $L_3$ and $L_4$), each from the base of the preceding one, resulting in the alternative arrangement of leaves on the main vine (V), while more and more roots (RT) are also being produced, x 1.5.**

The root and shoot primordia which are relatively undifferentiated at this time, lie in a cavity at the narrow end of the embryo. A longitudinal section through the flat surface of the embryo by the 7th day of moisture imbibition, reveals that the shoot apex is flanked by the leaf primordia which have begun to differentiate into young primordial leaves. The vascular strands of the fan-like single cotyledon have also become prominent and are all seen to be linked. These vascular strands probably serve to conduct nutrients arising from enzymatic digestion of the food materials of the endosperm, thereby making them available to the root and stem apices as their cells grow and further differentiate. These findings are similar in some respects to those reported by Lawton and Lawton (1967) for five species of *Dioscorea* including *D. rotundata*. But however, only one cotyledon was observed in our studies with *D. rotundata*, and this was prominent throughout the most critical phases of the germination process, with the vascular strands clearly visible and reflecting the cotyledon as being absorptive in nature. Although Lawton and Lawton (1967) claimed that there were two cotyledons in the seeds of the five species of *Dioscorea* that they worked with, they could not provide visual evidence for them.

The obvious presence of two leaf primordia, as revealed by anatomical sections made on the 7th day of seed imbibition in this study (Plate 2), is at variance with the report by Sadik and Okereke (1975) in which they observed only one primordium within the shoot axis of the *D. rotundata* embryo in the variety they studied. Their report, however, was not backed by any detailed internal morphological evidence.

As a result of the differential cell division among the tissues of the shoot apex, one of the leaf primordia elongated faster at the expense of the others, resulting in the production of the first photosynthetic leaf. A similar situation was also reported by Lawton and Lawton (1967) and Sadik and Okereke (1975). This differential growth of the leaf primordia is thought to be responsible for

the alternate leaf arrangement on the vine (stem) of the ensuing seedling from seed germination in this variety of *D. rotundata.*

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