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Full Length Research Paper

Behavior of various types of seeds of two species of yams tuber (*Dioscorea cayenensis* Lam. and *Dioscorea rotundata* Poir.) in Gabon

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Abstract

Low multiplication ratio of yam and scarcity of planting materials are major constraints militating against sustainable yam production. In order to evaluate the behavior of the four various types of seeds of two species of yams *Dioscorea cayenensis* and *Dioscorea rotundata,* cultivated on the experimental ground of the Higher National Institute of Agronomy and Biotechnology (INSAB), a test was realized in a randomized complete block design with six replications. The samples were cut and three levels of each tuber were used: proximal, medial and distal parts of the tuber. The fragments of tuber and the whole tuber represent the various types of seed used in this work. The results showed significant (P<0.05) differences in number of plants emerged and time of emergence in a mixture of 40% soil and 60% sand three months and half after planting. For all species, the proximal parts sprouted earlier than the medium parts and then the distal parts. The fragmentation of tubers in three (3) parts can show the existence of a gradient along the tuber in its potential for sprouting and growth. There was a highly significant (P<0.05) difference between yield performances after nine (9) months of culture. This technique improves the production of tubers in both species.

Keywords: *Dioscorea cayenensis*, *Dioscorea rotundata*, yams, seeding, tuber.

INTRODUCTION

Yams (*Dioscorea* spp.) are an annual tuber and monocotyledonous crop. The plant Genus comprises of over 600 species with only 10 species producing edible tuber. Six of these edible species are cultivated in Africa, West Indies, Asia, South and Central America (Amusa, 2000; Tamirou et al., 2008; Bousalem et al., 2010; Elsie, 2011; Petro et al, 2011; Ibitoye et al., 2013) and only three (3) of them are available in Gabon. The primary species cultivated are the white yam (*Dioscorea. rotundata),* yellow yam (*Dioscorea cayenensis)* and water yam (*Diosorea alata), D. rotundata* and *D. cayenensis* may have been first domesticated in the forest-savannah ecotone of West Africa (Hamon et al., 1995; Tostain et al., 2003).Yam tubers are important in different domains. Nutritionally, yams are a major source of nourishment to many populations in the world (Craufurd et al., 2006). Pharmaceutically, some species of *Dioscorea*, particularly *Dioscorea zingiberensis*, produces high concentration of diosgenin, a chemical used for the commercial synthesis of sex hormones and corticosteroids (Chen et al., 2003; Yuan et al., 2005; Islam et al., 2008). Agriculturally, yams tubers are used as planting material (Odjugo, 2008; Zannou, 2009). Yam also plays vital roles in traditional culture, rituals and religion as well as local commerce of African people (Izekor and Olumese, 2010). The conventional multiplication of *Dioscorea* species is by tuber seeds, a tuber fragment that grows and develops into a new tuber.

The absence of viable seeds, the long period required for obtaining usable tubers and phytosanitary problems are some of the factors that limit the rapid conventional propagation and economic exploitation of *Dioscorea* species (Balogun et al., 2006; Tschannen et al., 2005; Fotso et al., 2013). Yam production has been on the decline despite the increasing demand for local consumption and for export. Asumugha et al. (2009) reported that there is need for increased production of yam not only to satisfy domestic need but also export demand. The major constraint to increased production of yam in Africa is the scarcity of seed yam (Udealor and Ezulike, 2009), soil degradation, poor handling and storability, pest and disease and other environmental factors (Ibitoye and Attah, 2012) and large quantities of about 30% of the previous year's harvest are required (Okoli and Akoroda, 1995).To address this problem, the yam minisett technique has been developed as a quick and easy way of multiplying healthy seeds yams (Otoo et al., 2001).

The minisett technique involves the use of about 25 g setts to produce whole tubers which serve as seed yam (Okoli and Akoroda, 1995).The major problem militating against the adoption of the yam minisett technique by farmers is the low sprouting of minisett (Okoro, 2009; Ajieh, 2012).

They further reported that moist sawdust has been used effectively as a medium for sprouting minisetts but it is not easily available especially in areas where sawmills are not located (Asare-Bediako et al., 2007; Dasback et al., 2011). It has therefore been necessary to find substitutes for the sawdust. Yam cultivars differ in their duration to sprout and would respond differently under different sprouting media (Dasbak et al., 2011).The two Guinean yams (*D. cayenensis* and *D. rotundata*) were selected in this study because they are the most widely consumed in the study area. The land of culture zone has a sandy texture. Elsewhere, these yams do not produce tubers. Previous work on *in vitro* materials (Ondo Ovono et al., 2013) has revealed the possibility to increase the productivity by cutting the microtubers in three parts. The sprouting capacity of the eyes present in the different sections of the seed tubers can explain it, but there is little research to prove this assumption to be true (Zannou, 2009). The objective of the study was to examine the potential of using various types of seeds of two species of yams tubers (*D. cayenensis* and *D. rotundata*) in the study area, especially to produce seeds and use them as plants for propagation in conditions of Masuku, in Gabon.

MATERIALS AND METHOD

Site of the study

The study zone is located in the department of Mpassa, province of Haut-Ogooué, in the South–East of Gabon. It fits coarsely in a triangle, which co-ordinates are 13° and 14°20 East, and 1° and 1°45 South (Figure 1). It extends from the Congolese border in the East, on the Batékés plates, to the foot of the solid mass of Chaillu in the West, on approximately 6.960km^2. The altitude lies between 300 and 600 m. The climate is equatorial of "transition," characterized by a great dry season (April-August) and a great rain season (September-November), followed by two small seasons: the small dry season (December-February) and the small rain season (March-May). The presence of two dry seasons exerts an influence on the agricultural calendar, because they authorize two seasons of clearing and, thus two cycles of culture. The annual average temperature is 24°C. Annual average pluviometry varies between 1.700 and 2.200 mm. Annual relative humidity is 81% and passes by two minima in August-September and in March April (Guichard, 1977). The soil of Haut- Ogooué in general and Franceville in particular, was the subject of many works: Azzibrouck (1986); Chatelin (1966); Itongo (1998). The pH of the used zone varies between 4.5 and 5 and the content of clay is rather significant (45-50%). The pH determination of the soil by the Analysis laboratory of Soil of the Institut National Supérieur d'Agronomie et de Biotechnologies (INSAB), shows that the experimental site has an average potential acidity (pH water = 5.2 and pH KCl = 4.7). The vegetation is a secondary tropical forest having undergone several farming antecedents, in particular the culture of cassava.

Vegetable material

The experiments are based on two species of yams: *D. cayenensis* Lam., with yellow pulpit, and *D. rotundata* Poir., with white flesh, cultivated on the experimental ground of the INSAB.The common names are: Angankali for the yellow yam and Mva for the white yam. The choice of the tuber-mothers is done on the basis of the size and the shape of tubers for the two species. The classification of these yams is confused. In the older literature they are usually separated, and this approach is taken here, but most taxonomists now regard them as the same species.

These two yams are currently considered a botanical complex: the complex *Dioscorea cayenensis-D. rotundata,* because the species separation could not be upheld with the molecular and morphological data at hand (Hamon et al., 1992; Terauchi et al., 1992). They have whole sheets and no pubescent stems, more or less thorny, not winged generally not bulbiferous (Hamon et al., 1995). These two yams of West Africa origin (Terauchi et al., 1992) are cultivated on the same site during two crop years.

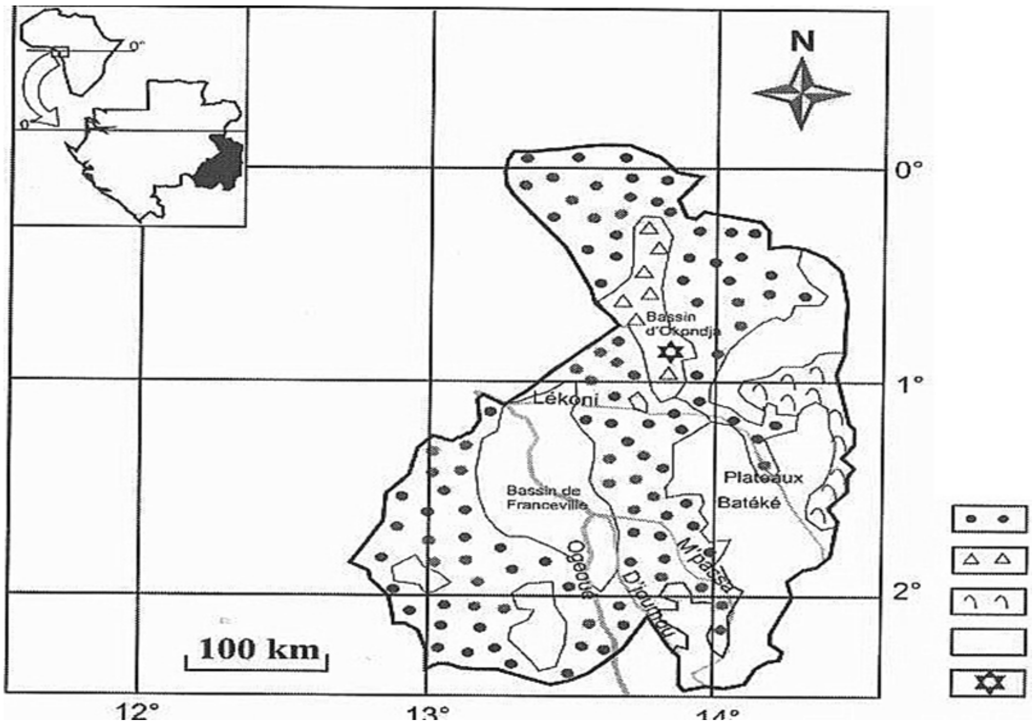

Figure 1: Area of study (Itongo, 1998)

Multiplication of the material

The tuber mothers of two local species of yams: *D. cayenensis* and *D. rotundata* taken in fields of farmers in Léconi in Haut- Ogooué, were used to constitute fragments. They were set up in September and October 2009 and harvested in July 2010. The samples were cut and three levels of each tuber were used: head for the proximal part, medium for the median part and tail for the distal part of the tuber. The fragments of tuber and the whole tuber represent the various types of the yam seed used in this work.

Preparation of the technical material

We used a mixture of sand and soil with the following proportions: 40% ground and 60% sand. Sand comes from the career of Bongoville in Haut-Ogooué, Gabon. The soil was taken near the near-by teaching pieces of INSAB. This substrate was selected because the two local species are only cultivated in the zone of Léconi, where the soil of the culture zone has a sandy texture. This mixture was used for the filling of braided artisanal bags, 30 cm in diameter, 60 cm depth. Three fragments were put in each bag, bags representing our experimental units. By bag, we put mixture to reach a 40 cm height. The bags, subjected to the conditions of the local environment, (temperature varying between 21° and 37 °C and relative humidity between 78 and 100%), are sprinkled each day, so as to maintain the humidity of culture media close to its capacity to the field.

Preparation of the yam seed

The knife used to cut the tubers as well as the table was disinfected after each cut with bleach diluted with the quarter. After cutting, the fragments of tubers were stored on the ground for healing during 24 hours. Then, the minisetts (fragments and whole tubers) were treated with a fungicide (Benlate, 6 g in 10 l of water) during 10 minutes, and then put to dry during 48 hours. 144 seeds were so used, 72 for each *Dioscorea* species, including 18 proximal parts, 18 medium parts, 18 distal parts and 18 whole tubers.

Culture and development

In order to evaluate the behavior of the four various types of seeds of the two species of yams, a test was realized: three similar fragments of tuber were put in a bag and each treatment was repeated six times for each species, the 48 experimental units thus obtained were arranged in a randomized complete block design with six replications

The yam minisetts inside the same bag were spaced from each other by 20 cm and the bags are spaced by 1 m. The seeds were completely buried to approximately 10 cm of depth. Yam stems being lianas, tutors are set put at each stem location.

Observations of the parameters of growth and development

To evaluate the growth of the plants, the observations were made every 7 days for the two assays. They consisted in measuring the following parameters: the number of seed tubers germinated, a tuber is regarded as having germinated, when the germ exceeds 5 mm in length. The rate of germination is calculated; the number of stems developed by each yam minisetts; the number of leaves developed on each stem; the average height of the stem (in cm) developed on each yam minisetts (measured using a decameter); the number of tubers formed in the bags, for each variety and type of yam minisetts for the two assays; the leaf area was evaluated. Leaf discs were punched out with a cork borer and the relationship between area and dry weight of the disc was used to estimate leaf area (Law-Ogbomo and Remison, 2008).

Determination of the fresh matter and dry matter

Fresh dry matters produced three months and half after the installation of the test were determined for each bag. The whole seedlings were taken by separating the aerial (stems and leaves) and the underground (root and tuber) parts. The leaves and the stems were weighed together. Concerning the underground part, after pulling it up, we determined the fresh and the dry weights of the tubers. The various parts were carried out to the drying oven during 48 h at 105° C, until obtaining a constant mass.

Determination of the average weight of the tubers at maturity

The average weight of the tuber at maturity, for each species, according to the type of the yam seed or fragment, was given with the last sampling.

Data Analysis

The final results are expressed in average value with the standard error on the average; the number of individual values (n) being specified in each case. The averages were separated by the test from Newman-Keul's (with the threshold of 5%).

RESULT

Germination

The observations carried out during the two assays indicated a very heterogeneous germination according to the species and the type of the yam seed. The percentage sprouting of yam minisetts and the whole tubers at 30, 60 and 90 days after the plantation, for the two species of yams are shown in Figure 2. At 60 days, for the species *D. cayenensis* (Figure 2A), only the distal part (tail) did not germinate. The rate of germination of the proximal part (head) was higher than 80%, while for the whole tuber it was less than 20%. At 90 days, all the types of seed - tubers germinated. The germination rate passed from 0% at 60 days to more than 83% at 90 days for the fragments of tuber resulting from the distal part. On day 90, 100% germination was observed for head and medium parts. For the species *D. rotundata* (Figure 2B), precocity (30% after 30 days) in the germination of the minisetts resulting from the proximal part was observed. 60 days were necessary to observe germination of the medium part and the whole tuber. After 90 days, the percentage of germination of the head fragments was superior to 80%, those of the medium part to 50%, those of the whole tubers to 33% and those of the tail to 5%. There was a significant interaction between the sprouting medium and the yam species.

Growth and development of the seedlings after three months and half

The growth of the stem continues with the appearance of the first leaves. The aerial part develops by emission of new stems. After three months and half of culture, we measured (Tables 1 and 2) the various parameters. All these parameters showed significant differences ($P<0.05$) between the types of yam minisetts at the threshold of 5%. For *D. cayenensis* (Table 1), the fresh weight of the aerial part lies between 83 g and 136 g per tuber or tuber fragment.

The leaf area of the seedlings resulting from the whole tubers is similar or higher than that of the seedlings from the other seed tubers. For *D. rotundata* (Table 2), the fresh weight of the aerial part varies from 52 g to 177 g depending on used fragment. The largest leaf area is also recorded at the seedlings resulting from the whole tubers. After three months and half of culture, only the species *D. rotundata* (Table 2) produced tubers. The weight of the tubers to harvest varies in a significant way. The sprouted yam minisetts resulting from the head gave the plants producing the largest tubers (73 g), followed by whole tubers (31g); the plants obtained from the distal part failed to produce a tuber.

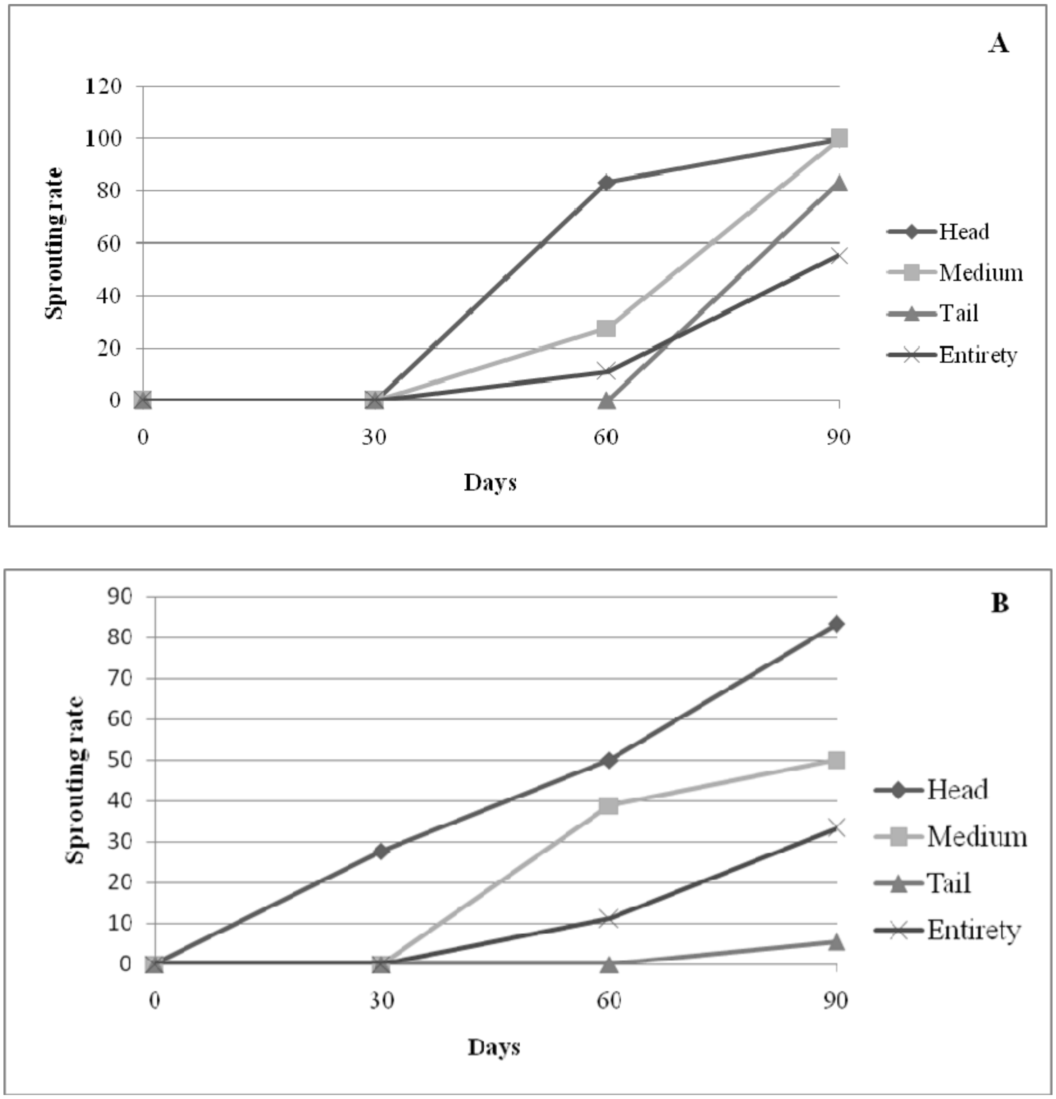

Figure 2: Evolution of the germination percentage of the sprouted yam minisetts of *Dioscorea cayenensis* (A) and of *Dioscorea rotundata* (B) according to culture time (n= 18).

Growth and development of the seedlings after 9 months

At maturity (Table 3), the number, the size and the weight of the tubers were measured on the two species for each fragment put in culture. For the species *D. cayenensis,* all the plants obtained produced 3 tubers each. On the other hand, for *D. rotundata,* the plants resulting from distal fragments of tubers produced more tubers (2.7) than those resulting from the proximal and median parts (2.3). There is no significant ($P<0.05$) difference between the types of the sprouted yam minisetts to the threshold of significance of 5%. The plants resulting from proximal fragments (head) produced tubers which size is approximately 15 cm for the two species, and those obtained from the distal part were longer. In term of tuber weight, plants resulting from heads of *D. cayenensis* produced the lightest, whereas the whole tubers of *D. rotundata* gave the plants producing the heaviest tubers.

DISCUSSION

The variation in sprouting of the minisetts in the sprouting medium was attributed to the characteristic differences among them in providing the required conductive environment for sprouting of the minisetts.This is a good prospect for the success of the minisett technology which is ideal for the production of seed yam to boost yam production. However, sometimes the failure of setts to

Table 1: Aerial part of *Dioscorea cayenensis* three months and half after planting (n=18)

Type of sprouted yam minisetts	**Stem number**	**Leaf number**	**Node number**	**Stem height (cm)**	**Leaf area (cm²)**	**Fresh weight of aerial part (g)**	**Dry weight of aerial part (g)**
Head	3.11±0.20^{a}	81.44±8.40^{a}	79.35±11.10^{a}	112.12±13.90^{c}	38.35±5.70^{ab}	136.48±12.90^{a}	40.24±7.70^{a}
Medium	3.03±0.60^{a}	59.32±5.70^{b}	58.44±6.90^{c}	129.67±20.40^{c}	37.67±6.90^{ab}	99.06±11.80^{ab}	29.64±8.90^{b}
Tail	3.16±0.90^{a}	46.07±7.10^{c}	46.21±4.21^{d}	181.55±15.50^{a}	34.16±4.40^{b}	82.94±7.70^{b}	25.45±6.20^{b}
Entirety	2.21±0.30^{b}	46.17±5.20^{c}	64.55±7.30^{b}	152.52±13.60^{b}	41.35±8.80^{a}	103.12±8.60^{ab}	29.95±5.50^{b}

The average numbers followed by identical letters are broadly equivalent to the probability threshold $p < 0.05$

Table 2: Aerial and underground parts of *Dioscorea rotundata* three months and half after planting (n=18)

Type of sprouted yam minisetts	**Stem number**	**Leaf number**	**Node number**	**Stem height (cm)**	**Leaf area (cm²)**	**Fresh weight of aerial part (g)**	**Dry weight of aerial part (g)**
Head	3.11±0.20^{a}	81.44±8.40^{a}	79.35±11.10^{a}	112.12±13.90^{c}	38.35±5.70^{ab}	136.48±12.90^{a}	40.24±7.70^{a}
Medium	3.03±0.60^{a}	59.32±5.70^{b}	58.44±6.90^{c}	129.67±20.40^{c}	37.67±6.90^{ab}	99.06±11.80^{ab}	29.64±8.90^{b}
Tail	3.16±0.90^{a}	46.07±7.10^{c}	46.21±4.21^{d}	181.55±15.50^{a}	34.16±4.40^{b}	82.94±7.70^{b}	25.45±6.20^{b}
Entirety	2.21±0.30^{b}	46.17±5.20^{c}	64.55±7.30^{b}	152.52±13.60^{b}	41.35±8.80^{a}	103.12±8.60^{ab}	29.95±5.50^{b}

The average numbers followed by identical letters are broadly equivalent to the probability threshold $p <0.05$

Table 3: Tuber growth of *Dioscorea cayenenesis* and *Dioscorea rotundata* 9 months after planting (n=18)

Type of the sprouted yam minisetts	***Dioscorea cayenensis***			***Dioscorea rotundata***		
	Average length of tubers (cm)	Average weight of tubers (g)	Average number of tubers	Average length of tubers (cm)	Average weight of tubers (g)	Average number of tubers
Head	15.47±0.88^{b}	146.90±18.58^{b}	3.33±0.80	15.16±1.03^{b}	160.28±38.32^{b}	2.33±0.55
Medium	15.55±1.53^{b}	186.94±30.36^{a}	3.40±1.10	14.42±0.89^{c}	130.00±18.36^{c}	2.33±0.55
Tail	17.41±1.43^{a}	197.16±31.60^{a}	3.10±0.90	18.93±6.61^{c}	101.75±12.62^{d}	2.66±0.66
Entirety	17.97±0.98^{a}	189.38±22.76^{a}	3.20±0.80	15.71±1.45^{b}	194.37±33.42^{a}	2.50±0.70

The average numbers followed by identical letters are broadly equivalent to the probability threshold $p < 0.05$

sprout uniformly or delay in sprouting due to dormancy of tubers hampers the effective use of this technology. In the current study, it was observed that minisetts from different portions of the yam responded differently to the sprouting medium. The significant variation in sprouting of the minisetts among yam cultivars was attributed to genotypic differences and it agrees with an earlier report by Ikeorgu and Ogbanna (2009). For all species, the proximal part sprouted earlier than the medium parts and then the distal parts. These results are in contrast with those obtained by Ndzana et al. (1992) and Assembe (2009). Ndzana et al. (1992) reported that the middle section of yam sprouted uniformly and had the highest number of sprout. Assembe (2009) showed that for the species *D. rotundata* it is the medium part of the tuber which has the highest germination, followed by distal part and, finally, by the proximal part. And, for the species *D. cayenensis,* the distal part has the highest germination rate, followed by medium and, finally, proximal part.These results indicate that tubers from middle-derived setts store starch at higher contents relative to those from head and tail sections. The low sugar content implies that starch is being stored rather than degraded in tubers from middle-derived setts, an attribute that is desirable in yams (Wheatley et al., 2002). In all the cases, an active gradient of germination from the head towards the base of the seed mother has been identified in the majority of the yam varieties (Mathurin and Degras, 1981), in particular in the Krengle variety (Dumont and Topka, 1990; Zoundjihekpon et al., 1995).

The proportion of non- emerged plants after planting was highest and yields the lowest when the distal part was used as planting material. However, there were some variations among species (Zannou et al., 2009).The results suggest that there could be a complex genetic – physiological property governing the sprouting ability of each fragment of the tubers. In addition, as revealed by other studies, along the tuber, there could a gradient of earliness in sprouting, in the availability of nutrient reserves and the ability which decreases from the proximal to the distal part (Kossou, 1990).Sprouting ability and biochemical constituents differ with the physiological regions and are higher in apical than basal regions of *D. rotundata* (Jaleel et al., 2008). On *D. cayenensis* cultivars, the biochemical properties and active starch content are significantly higher in middle-derived tubers than tail-derived setts (Wheatley et al., 2002). Also, the emergence and yield are significantly influenced by both the sprouting state at planting and the origin of a sett with respect to its position on the mother tuber (Tschannen et al., 2005), conducting them to suggest that limited resources should be to directed to apical setts and sprouted which have the highest yield potential for *D. cayenensis* and *D. rotundata*. This result may suggest that cultivars of *Dioscorea* species were originally generated by different ancestors of yam in the past. This is also possible since genetic diversity of wild yams is structured geographically (Tostain et al., 2003). The differential responses of species and cultivars suggest that the behavior of various types of seed could be used to distinguish early and late maturing varieties of yams (Shiwachi et al., 2002). *Dioscorea rotundata* is the early maturing variety with a short vegetative cycle while *Dioscorea cayenensis* is late maturing variety, with a long vegetative cycle. *D. cayenensis* types mature in 10-12 months and *D. rotundata* in 7-8 months. The early maturing of the latter permits double harvesting, as the early tuberization results in large, though immature tubers being present after 4 months. *D. cayenensis* is relatively tolerant of sandy soils; *D. rotundata* thrives best on heavy soils even with high clay content.

We easily obtained the tuberization of *D. cayenensis* and *D. rotundata* nine months after planting. The highest number of tubers per plants (2 to 3), the greatest tuber size (17 to 18 cm) and higher fresh weight of tuber (194.37 to 197.16 g), are proofs of a good technic. This can be partly explained by the fact that, naturally, *Dioscorea cayenensis* and *Dioscorea rotundata* grows and develops well in the regions where the soil is mixed with sand (Degras et al., 1977; Ngo Ngwe, 2009).The significant interaction between the sprouting medium and the yam cultivars implies the need for careful selection of sprouting medium for a particular yam cultivars or varieties. The percentage success of the various yam species indicated how easier or difficult working on the particular species as far as minisetts production is concerned.The relationship between the aerial parts of the plantlet and the tubers formed would be important to specify and understand the mechanisms of transport of assimilation towards the areas of filling of tubers as recommended by Zinsou (1998). Propagation by seed is possible on all portions (head, middle and tail) of freshly harvest tubers and could be used to induce multiple tuber formation in yams. Seed production is irregular; some cultivars do not sett seed. Seedlings are weak and require careful attention in the nursery. *Dioscorea rotundata* exhibited greater potential for success in the minisett technique of seed yam production and are recommended for adoption in the area of study.

CONCLUSION

This study showed that germination of the fragments of tubers is thus possible in a mixture of soil and sand with the following proportions: 40% soil and 60% sand. The development of this technology can be used to resolve the low production of yam seeds in our region and satisfy the demands from farmers. The major obstacles with the expansion of this technique are the weak rate of multiplication of the plantation material and the disease proliferation. Further studies are needed to evaluate the performance of plants transferred to the fields.

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Author's contribution

The present study was conducted at the University of Masuku, Gabon. P. Ondo Ovono was the coordinator of this study and collected all data. He discussed the results with C. Kevers and they wrote the manuscript in the present form. J. Dommes revised the manuscript.

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