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PRODUCTION OF SEED TUBERS FROM THE AERIAL STEMS OF THE YAM SPECIES DIOSCOREA ALATA (L.) AND DIOSCOREA CAYENENSIS-ROTUNDATA (L. & P.) IN CÔTE D'IVOIRE

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ABSTRACT

The aim of this work is to produce at field the high quantities of yam tuber-seeds of good quality by vitroplants regenerated from the cuttings of aerial stems. Tuber-seeds of ten cultivars of yam whose five of *Dioscorea alata* (cDa083, cDa053, cDa115, cDa150 and cDa266)) and five of *Dioscorea cayenensis-rotundata* (cDr015, cDr027, cDr150, cDr206 and cDr148) showing distinct agro-morphological characteristics were used as vegetable material for the production of the aerial stems in greenhouse. The vitroplants obtained starting from primary explants taken on older aerial stems of 14 and 21 days, underwent indirect weaning before being transplanted at field on the ground added or not with the chicken excrement. Each plant produced on average 5 to 7 tubers of 4 to 7 kg with the yields at hectare ranging 38 to 67 tons. These vitroplants produced large seed tubers in large quantities in the field. In vitro cultivation makes it possible to obtain the seeds needed for high yam profitability and to safeguard all genetic resources by conserving them in the form of in vitro plants.

Keywords : *yams, explants, vitroplants, multiplication, weaning, tuber-seeds.*

RÉSUMÉ

Production de tubercules-semence à partir des tiges aériennes des espèces d'igname Dioscorea alata (L.) et Dioscorea cayenensis-rotundata (L. & P.) en Côte d'Ivoire

L'objectif de ce travail est de produire au champ de grandes quantités de tubercules-semences d'igname de bonne qualité par des vitroplants régénérés à partir de boutures de tiges aériennes. Des tubercules-semences de dix cultivars d'igname dont cinq de Dioscorea alata (cDa083, cDa053, cDa115, cDa150 et cDa266)) et cinq de Dioscorea cayenensis-rotundata (cDr015, cDr027, cDr150, cDr206 et cDr148) présentant des caractéristiques agro morphologiques distinctes ont été utilisés comme matériel végétal pour la production des tiges aériennes en serre. Les vitroplants obtenus à partir d'explants primaires prélevés sur des tiges aériennes âgées de 14 et 21 jours, ont subi un sevrage indirect avant d'être transplantés au champ sur le sol additionné ou non fiente de poulet. Chaque plant a produit en moyenne 5 à 7 tubercules, de 4 à 7 kg avec des rendements à l'hectare allant de 38 à 67 tonnes. Ces vitroplants ont produit de gros tubercules-semence en grande quantité au champ. La culture in vitro permet d'obtenir les tubercules-semences ayant une rentabilité élevée de l'igname et de sauvegarder toutes les ressources génétiques en les conservant sous forme de vitroplants.

Mots-clés : *igname, explants, vitroplants, multiplication, sevrage, tubercules-semences.*

I - INTRODUCTION

Yams (Dioscorea spp) are herbaceous plants with a tuber and monocotyledonous angiosperms that multiply vegetatively through the tuber. They belong to the family Dioscoreaceae and the genus Dioscorea [1]. The yam, by its global production estimated at about 60.2 million tons per year, occupies the 4th place in the world, after potato, sweet potato and cassava [2]. In Côte d'Ivoire, yam (Dioscorea spp) ranks first in food crop production. Its annual production is estimated at 7 148 000 tons of fresh tubers ahead of cassava [2]. Tubers are food sources for millions of people and serve as a staple food for 2/3 of the Ivorian population. Tubers used as planting material are cut into fragments to form seedlings or seed tubers for the next planting. They are a source of income for producers and traders [3]. Despite yam's leading position among food crops, its annual production is still insufficient to meet the food needs of Côte d'Ivoire's rapidly growing population [4]. One of the major causes is the lack of seedlings or planting material due to the use of more than 1/3 of tubers produced for consumption as planting material. It takes more than 2 tons of tubers to plant one hectare of yam. Also, for the planter starting a first plantation, the cost of acquiring seedlings is high and constitutes about 33 to

40 % of the total cost of production. The low yield is also due to the use of unimproved local varieties and the lack of access to the few improved seeds. The need for seedlings considerably reduces the quantity of tubers intended for consumption. After the creation of new yam plantations, there is a famine in the farming community due to the unavailability of edible tubers. To solve this seed problem, the technique of mini fragments or mini setts has been developed in Nigeria for a massive and rapid production of yam seeds [5]. [6, 7] have succeeded in producing yam seedlings from cuttings of aerial stems. However, the size of the tubers was very small, so they had to be replanted over one or two cycles to obtain suitable seedlings for use in the field. The methods of production of yam seedlings by vegetative multiplication developed by some researchers do not allow, a priori, to obtain seeds of good sanitary quality. It is therefore necessary to develop the technique of vegetative multiplication of yam by in vitro culture which presents many advantages and to find a plant material other than tubers to produce seeds. In vitro culture covers all plant material grown under aseptic conditions. It covers all plant material cultivated under aseptic conditions. It uses the genetic potential of plant cells. These cells contain all the genetic information of a plant in their nuclei, and should therefore be able to reproduce that plant [8]. The general objective of this study is therefore to produce large tuber-seeds of yam of good quality in large quantities in the field from in vitro plants derived from aerial stems in a single cycle of culture.

II - MATERIAL AND METHODS

II-1. Study Site

The trials were carried out at the Centre National de Recherche Agronomique (CNRA) located in Abidjan at Km 17 in Adiopodoumé. The geographical coordinates of the center are between 5˚7'N latitude and 3˚31'W longitude. The climate is equatorial. Temperatures vary between 27˚C and 35˚C on average. Annual rainfall is 2000 mm [9].

II-2. Material

The plant material used was composed of ten cultivars belonging to two yam species. Five cultivars (cDa083 or Florido; cDa053 or C18; cDa115; cDa150 and cDa266 or Kanga-nza) belonged to the Dioscorea alata species and the other five cultivars (cDr015 or C20 ; cDr027 or Krenglè; cDr150 or Krenglè doko tangbi; cDr206 or Kponan and cDr148 or Lokpa) belong to the Dioscorea cayenensis- rotundata species. These cultivars are the most widely grown and consumed by farmers. Cultivars C18 and C20 are improved varieties. In *D.*

alata, cultivars cDa083, cDa115 and cDa150 belong to the Nza variety, while cultivars cDa053 and cDa266 are of the Bètè-bètè variety. With regard to *D. cayenensis-rotundata*, cultivars cDr015, cDr027 and cDr150 are late yams harvested once, while cultivars cDr206 and cDr148 are early yams harvested twice a year. All cultivars have different agro-morphological characteristics that distinguish them from one another. Tubers from these cultivars used for greenhouse stem production come from the research station of the Centre National de Recherche Agronomique (CNRA) in Bouaké. Yam stem cuttings taken in the greenhouse were therefore used to initiate in vitro cultivation in order to preserve the yam cultivars in the vitro library in the form of in vitro plants. The vitroplants of selected and propagated cultivars were weaned and transferred to the field for the production of seed tubers of yam.

II-3. Methods

II-3-1. Greenhouse yam stalk production

Tuber fragments or seedlings weighing around 200-300 g were placed in nursery bags 20 cm long and 10 cm wide, or in pots 6 cm in base diameter and 15 cm high, filled with black soil. This soil was taken from the undergrowth of a forest and forms a humus-bearing surface layer. This soil was kept in the greenhouses for stem production. The perimeter of the greenhouse was netted to allow water and air to pass through. Depending on the level at which the fragments were removed from the tuber, 3 batches of seedlings were made up : fragments from the base or distal part, fragments from the middle or medial part and fragments from the top or apical part of the tuber (figures 4). The nurseries were watered every two days to facilitate fragment rooting. Watering was carried out for two weeks, at a rate of one liter of water per bag. The parameters studied were germination rate, time to germination, number of stems and number of nodes per sampling level.

II-3-2. Sterilization of primary explants

Primary explants or cuttings taken from the greenhouse were rinsed with sterile distilled water before being introduced into the culture room. They were rinsed again with sterile distilled water for 5 min before being subjected to various disinfectants in a laminar flow hood (Thermo Scientific, Heraguard ECO 1.8/95). Primary explants were sterilized in the following solutions : soaking in 70 % (v/v) ethanol for 5 minutes, then rinsing 3 times with sterile distilled water for 5 minutes, immersion, for 15, 20 or 30 minutes, in a 2 % or 3 % aqueous calcium hypochlorite solution to which 2 drops of tween 20 have been added, then rinsed 3 times with sterile distilled water for 5 minutes. At the end of sterilization, the cuttings were dried before culturing.

II-3-3. Regeneration of primary explants

Sterilized, necrotic primary explants aged 14 to 21 days were grown on the basic initiation medium of [10] macroelements, microelements and NITSCH vitamin solution. The pH was adjusted to 5.8 with 1N HCl and 1N NaOH. 2 $g.l^{-1}$ activated charcoal, 30 $g.l^{-1}$ sucrose and 8 g/l agar were added to this basic medium (MS). The medium was distributed in test tubes (16 mm x 150 mm) at a rate of 10 ml/test tube and autoclaved at 120°c for 20 minutes at a pressure of 1 bar. A single cutting is planted vertically with the node exposed above the culture medium in each test tube. The test tubes, tightly closed with plastic caps and sealed with cellophane paper, were placed in a culture chamber or conditioning room at a temperature kept constant at 27°c and a photoperiod of 12 hours. The room's relative humidity was kept constant at 80 %. The growth chamber was illuminated at 2000 lux by 36 W T8 white fluorescent lamps 118 cm long (Ingelec T8G).

II-3-4. Multiplication of secondary explants

Secondary explants or microcuttings were cultured on basic media with or without the addition of phytohormones (cytokinins and auxins) at various concentrations ranging from 10-4 mol. l^{-1} to 10-6 mol. l^{-1} The auxins used were Naphthalene Acetic Acid or NAA and 2- 4 Dichlorophenoxy acetic acid or 2- 4 D at 10-6 mol. l^{-1}, to promote rhizogenesis. The cytokinins used were Benzyl Amino Purine (BAP) and Kinetin or Kin, to activate caulogenesis and axillary budding.

II-3-5. Transplantation of vitroplants in the field

The vitroplants removed from the test tubes and the agar were transferred directly to field ridges. The ridges were 5 to 8 m long, 60 cm wide, 80 cm high and 100 cm apart. The ridges were placed under 150 cm-high shades. The vitroplants, 60 cm apart, were watered every morning between 6 a.m. and 7 a.m. for 2 weeks. This resulted in 10,000 vitroplants per hectare. The vitroplants were planted either on unamended forest soil, or on forest soil enriched with organic fertilizer made from chicken droppings at a rate of 150 g/m^2.

II-3-6. Agronomic variables Measured

Variables were selected from the yam descriptor [6]. These variables were quantified in the field. The variables studied were : survival rate of vitroplants in the field; mean number of tubers per in vitro plant; mean

tuber mass per in vitro plant; mean tuber mass et yield per hectare. The yam tuber yield (Y), the Yield of a primary explant and the quantity of tubers was calculated using the following ***Formula*** :

$$Y = M \times d \quad (1)$$

Y = Yield per hectare ; M = Mean mass of the tubers of a vitro plant ; d = density = number of in vitro plants per hectare.

$$Y\ 1ep = Y \times S\ 1ep \quad (2)$$

Y 1ep = Yield of a primary explant; Y = Yield per hectare; S 1ep = Number of cultivable hectares per primary explant after one year of propagation.

$$Qh = N \times d \quad (3)$$

Qh = Quantity of tubers (seeds) per hectare; N = Mean number of tubers per plant; d = density per hectare.

$$Q_{1ep} = Qh \times S\ 1ep \quad (4)$$

Q_{1ep} = Quantity of tubers obtained from a primary explant.

II-4. Data analysis

Cultivars and substrate type were subjected to analysis of variance (ANOVA) to determine their individual or combined effects on yield variables and yam vitro plant yield. When a significant difference was observed between the different factors, Least Significant Difference (LSD) multiple range-tests procedure were used to separate the means of the different treatments. Means were given as mean followed by standard deviation (M ± SD). Significant differences were determined at $P \leq 0.05$, [11]. All statistical analyses were performed using R software, version 4.1.2. [12].

III - RESULTS

III-1. Combined effect of growing substrates and cultivars on yield variables of yam in vitroplants

For all yam cultivars, the results of the analyses showed a highly significant difference between the values of the yield variables of vitroplants grown on soil supplemented with chicken droppings and those of vitroplants grown on soil without fertilizer ***(Table 1)***. For each cultivar, these yield variables and the yield of vitroplants grown on soil fertilized with chicken droppings had higher values than those of vitroplants grown on soil without chicken droppings. With

the exception of cultivars cDa150 and cDa266, all cultivars showed significant differences in mean tuber mass between in vitro plants grown on soil fertilized with chicken droppings and those grown on unfertilized soil. The substrate of soil supplemented with chicken droppings thus resulted in higher yam in vitro plant production than the unfertilized soil ***(Table 1 and Figures 1 -4)***.

Table 1 : *Yield variables values of yam plants in the field by growing medium*

Performance variable	Soil + chicken droppings	Unfertilized soil	Fisher Test	
			F	P
Mean number of tubers per plant	5.38 ± 1.52^{a}	3.85 ± 1.25^{b}	115.84	< 0.05
Total mass of tubers per plant (grams)	3472.35 ± 2245^{a}	2494.50 ± 1497^{b}	42.95	< 0.05
Mean mass of a tuber (grams)	636.35 ± 385.50^{a}	634.75 ± 335.39^{a}	2.66	0.103
Yield per hectare (tons)	34.73 ± 26.93^{a}	24.94 ± 17.96^{b}	42.95	< 0.05

Values followed by the same superscript in a line were not significantly different $P \geq 0.05$.

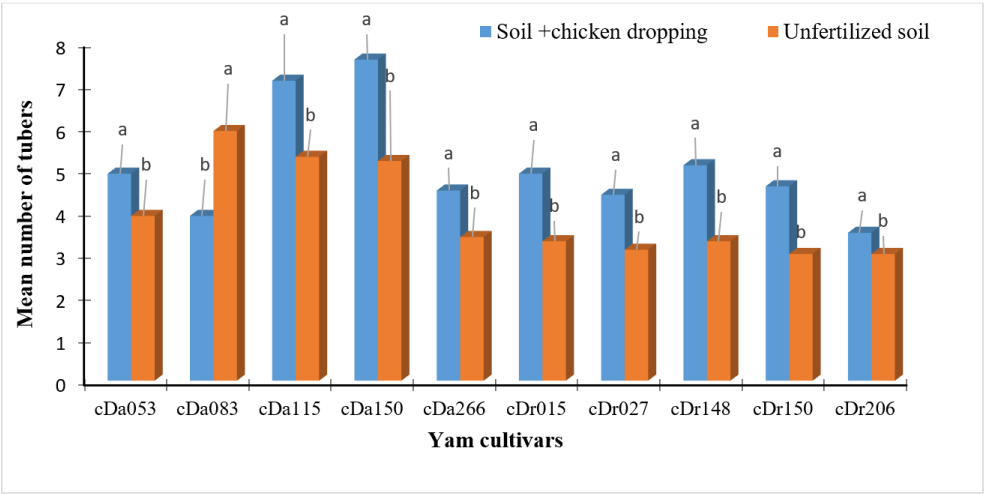

Figure 1 : *Mean number of tubers produced by yam vitroplants in the field, by type of growing medium*

Values followed by the same superscript in a line were not significantly different $P \geq 0.05$. Cultuvars of Dioscorea alata (cDa083 or Florido; cDa053 or C18 : improved varietie, cDa115 : Nza variety, cDa150 : Nza variety, cDa266 or Kanga-nza). Cultivars Dioscorea cayenensis-rotundata (cDr015 or C20 : improved varietie, cDr027 or Krenglè; cDr150 or Krenglè doko tangbi; cDr206 or Kponan, cDr148 or Lokpa).

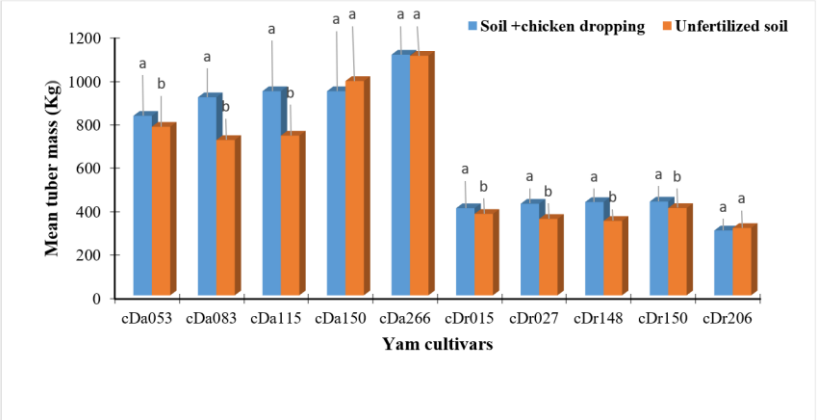

Figure 2 : *Mean tuber mass produced by yam vitroplants in the field according to type of growing medium*

Values followed by the same superscript in a line were not significantly different P ≥ 0.05. Cultuvars of Dioscorea alata (cDa083 or Florido; cDa053 or C18 : improved varietie, cDa115 : Nza variety, cDa150 : Nza variety, cDa266 or Kanga-nza). Cultivars Dioscorea cayenensis-rotundata (cDr015 or C20 : improved varietie, cDr027 or Krenglè; cDr150 or Krenglè doko tangbi; cDr206 or Kponan, cDr148 or Lokpa).

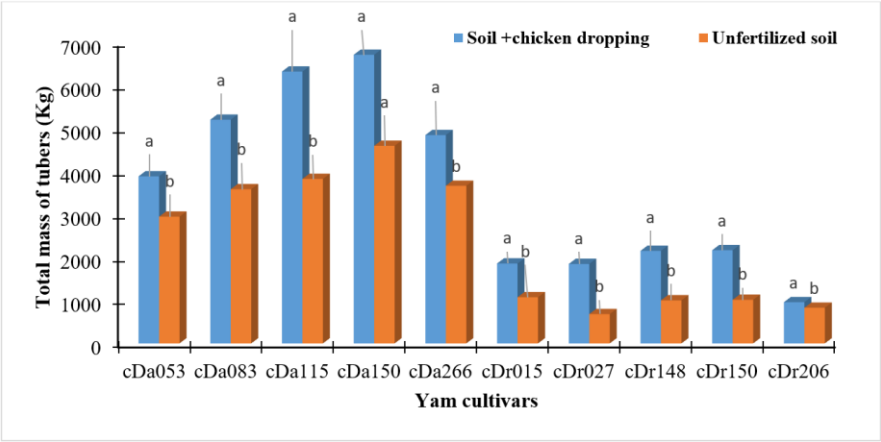

Figure 3 : *Total mass of tubers produced by yam vitroplants in the field, by type of growing medium*

Values followed by the same superscript in a line were not significantly different P ≥ 0.05. Cultuvars of Dioscorea alata (cDa083 or Florido; cDa053 or C18 : improved varietie, cDa115 : Nza variety, cDa150 : Nza variety, cDa266 or Kanga-nza). Cultivars Dioscorea cayenensis-rotundata (cDr015 or C20 : improved varietie, cDr027 or Krenglè ; cDr150 or Krenglè doko tangbi; cDr206 or Kponan, cDr148 or Lokpa).

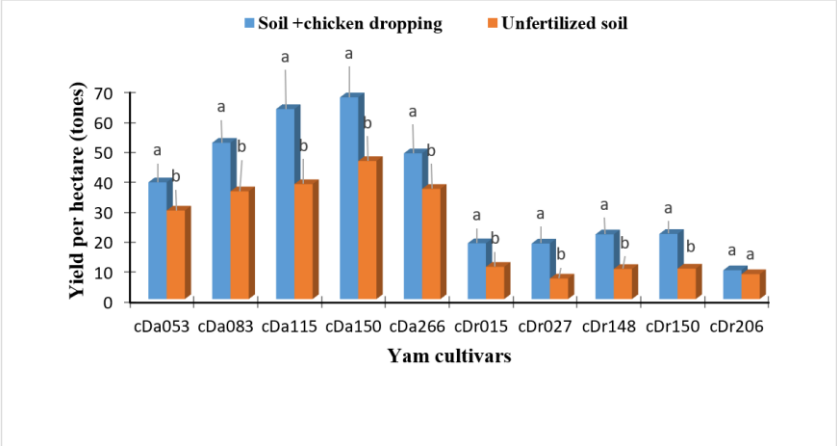

Figure 4 : *Average yield per hectare (in tons) of yam vitroplants by type of growing medium*

Values followed by the same superscript in a line were not significantly different P ≥ 0.05. Cultuvars of Dioscorea alata (cDa083 or Florido; cDa053 or C18 : improved varietie, cDa115 : Nza variety, cDa150 : Nza variety, cDa266 or Kanga-nza). Cultivars Dioscorea cayenensis-rotundata (cDr015 or C20 : improved varietie, cDr027 or Krenglè; cDr150 or Krenglè doko tangbi; cDr206 or Kponan, cDr148 or Lokpa).

III-2. *Yield of vitroplants of different yam cultivars subjected to indirect weaning and grown in the field on soil supplemented with chicken droppings*

The values of the yield variables for vitroplants of the different yam cultivars subjected to indirect weaning and grown in the field on soil supplemented with chicken droppings are presented in ***Table 2***. For all these yield variables, there was a highly significant difference between cultivars. *Dioscorea alata* cultivars generally produced more tubers than *Dioscorea cayenensis-rotundata* cultivars. The average number of tubers per plant ranged from 4.9 to 7.6 in *D. alata* cultivars and from 3.5 to 4.9 in *D. cayenensis- rotundata* cultivars ***(Figure 5)***. Cultivar cDa150 produced the highest number of tubers (7.6), while the lowest average number of tubers (3.5) was observed in cultivar cDr206. The total mass of tubers produced per vitro plant was higher in *D. alata* cultivars (6,720 to 3,890 g per plant) than in *D. cayenensis- rotundata* cultivars (960 to 2,170 g per plant). The highest value (6,720 g or 6.72 kg /plant) was obtained in cultivar cDa150, while cultivar cDr206 had the lowest value (960 g or 0.96 kg/ plant). Statistical analyses showed significant differences in mean tuber mass between cultivars of the two yam species. For *D. alata*, average tuber mass ranged from 1108.08 g for cultivar cDa266 to 826.5 g for

cultivar cDa053. For *D. cayenensis-rotundata*, average tuber weight ranged from 431.58 g for cultivar cDr150 to 298.58 g for cultivar cDr206. *D. alata* cultivars gave the best yields per hectare compared with *D. cayenensis-rotundata*. For *D. alata*, *yields* ranged from 38.90 tons/hectare for cultivar cDa053 to 67.2 tons/hectare for cultivar cDa150, while for *D. cayenensis-rotundata*, yields ranged from 9.60 tons/hectare for cultivar cDr206 to 21.70 tons/hectare for cultivar cDr150. ***Figure 6*** shows the variability in tuber size produced by vitroplants of the different cultivars.

Table 2 : *Comparison of yield parameters of vitroplants of different yam cultivars in the field*

Yam cultivars	Mean number of tubers per plant	Total tuber mass per plant (grams)	Mean mass of a tuber (grams)	Yield per hectare (tons)
cDa053	4.9 ± 1.09^{c}	3890 ± 823.08^{d}	826.5 ± 202.8^{c}	38.90 ± 9.8^{d}
cDa083	5.91 ± 1.26^{b}	5208 ± 1193.31^{b}	911.76 ± 192.76^{c}	52.08 ± 14.31^{b}
cDa115	7.1 ± 1.6^{a}	6330 ± 283.86^{b}	939.97 ± 268.7^{b}	63.30 ± 10.21^{b}
cDa150	7.6 ± 2.13^{a}	6720 ± 1532.85^{a}	940 ± 380.21^{b}	67.20 ± 18.39^{a}
cDa266	4.5 ± 0.99^{c}	4855 ± 1141.66^{c}	1108.08 ± 277.16^{a}	48.55 ± 13.69^{c}
cDr015	4.9 ± 1.16^{c}	1860 ± 798.56^{e}	401 ± 259.54^{e}	18.60 ± 9.58^{e}
cDr027	4.4 ± 0.91^{c}	1850 ± 444.87^{e}	421.5 ± 96.12^{b}	18.50 ± 5.33^{e}
cDr148	5.1 ± 1.2^{b}	2155 ± 623.49^{e}	429.5 ± 153.77^{d}	21.55 ± 7.48^{e}
cDr150	4.6 ± 0.95^{c}	2170 ± 523.21^{e}	431.58 ± 141.07^{d}	21.70 ± 6.27^{e}
cDr206	3.5 ± 1.03^{d}	960 ± 283.14^{f}	298.58 ± 91.27^{f}	9.6 ± 3.4^{f}
F	17.21	54.12	42.95	54.12
P	< 0.05	< 0.05	< 0.05	< 0.05

Values followed by the same superscript in a column were not significantly different $P \geq 0.05$. Cultuvars of Dioscorea alata (cDa083 or Florido; cDa053 or C18 : improved varietie, cDa115 : Nza variety, cDa150 : Nza variety, cDa266 or Kanga-nza). Cultivars Dioscorea cayenensis-rotundata (cDr015 or C20 : improved varietie, cDr027 or Krenglè; cDr150 or Krenglè doko tangbi; cDr206 or Kponan, cDr148 or Lokpa).

Figure 5 : *In vitro plant tubers at harvest*

(A) : Dioscorea cayenensis-rotundata ; (B): Dioscorea alata

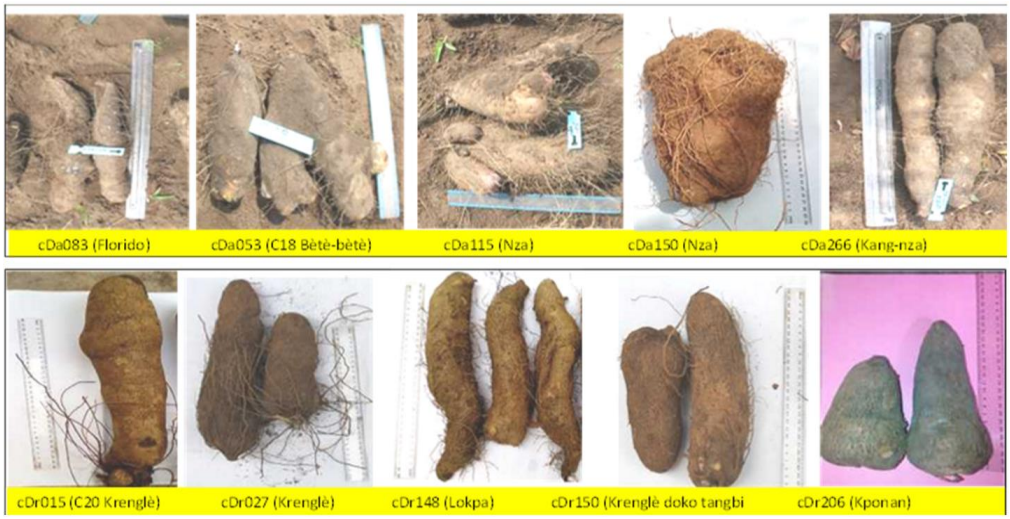

Figure 6 : *Tubers produced by vitroplants of different yam cultivars in the field*

IV - DISCUSSION

The aim of this study was to find plant material other than tubers for seed production. Thus, primary explants from stems were selected for in vitro cultivation of cultivars. Primary explants taken from stems aged between 14 and 21 days were the best for regeneration of all cultivars of the two yam species *D. alata* and *D. cayenensis-rotundata*. The average number of nodes was higher than with primary explants taken from stems aged 14 and 21 days.

These results could be explained by the fact that, on sterilized culture media, not only are primary explants relatively easy to disinfect, but they are also better suited to organogenesis than leaves, tubers and roots, as observed by [13 - 15]. This better reaction of primary explants taken from stems aged 14 and 21 days could be explained by the greater reactivity of the youngest explants. Similar observations were made by [16] during his studies on germination of microtubers from knotted cuttings of yam plants. Yam tubers were harvested 9 months after transferring the vitroplants to the field. Results showed that yield variables and tuber yield were better for all cultivars when grown on soil supplemented with chicken droppings. *D. alata* cultivars produced an average of 5 to 7.5 tubers per vitro plant, with an average tuber weight of between 830 g and 1,110 g. Yields per hectare ranged from 39 to 67 tonnes. As for *D. cayenensis-rotundata* cultivars, the average number of tubers per plant is 3.5 to 5, with an average tuber weight ranging from 298.5 to 430 g. Their yield per hectare then ranges from 9.6 to 21.7 tonnes. This result could be explained by the availability of minerals in the cultivation soil. In fact, chicken droppings buried in the soil, after decomposition, enriched the soil with nitrogen, phosphorus, potassium, calcium and trace elements.

Once decomposed, these minerals are used by the yam plants to ensure their growth and development. Also, as manure decomposes slowly, these elements will be available to yam plants throughout their cycle. Organic fertilizers are known for their slow release of nutrients when applied to the soil [17]. Nitrogen, as a component of photosynthetic pigments, in particular chlorophylls, is essential for plant growth and tuber formation, thanks to the synthesis of carbohydrates via photosynthesis. It is also involved in the synthesis of proteins, especially enzymes, without which no metabolic activity can take place in living organisms. Phosphorus is involved in the synthesis of chlorophylls and proteins. It also plays a role in cell division and, consequently, in plant growth. Potassium, an important constituent of plant cells, influences water absorption by roots. It plays a role in plant respiration and photosynthesis. All in all, these three minerals positively influence the vital plant activity of photosynthesis. Indeed, it is through photosynthesis that carbon is incorporated into the plant and contributes to the synthesis of plant organic compounds [18]. These results differ from those of [19 - 21], who worked on other *D. alata* cultivars and obtained an average of 1.5 to 2.5 tubers per vitroplant, with an average tuber weight of 155 to 200 g. [22] obtained even lower yields by producing mini-tubers from aerial stem fragments grown on carbonized rice husk in the first crop cycle

V - CONCLUSION

The aim of this study was to produce large quantities of yam seed tubers in the field from vitroplants grown from aerial stems in a single crop cycle. Field production of seed tubers from cultivars of the two yam species, *D. alata* and *D. cayenensis-rotundata*, has been improved by the use of seed preserved in the form of vitroplants.Healthy vitroplants from primary explants harvested from 14- and 21-day-old stems of all cultivars and regenerated in modified MS medium in less than a week served as secondary explants for seed tuber production.These vitroplants produced large, high-quality seed tubers in large quantities in the field. Weaned vitroplants transplanted to the field on soil amended with chicken droppings produced large tubers, sometimes reaching over 3.5 kg. On average, these vitroplants produced more tubers, 5 to 7 per plant, with an average tuber weight of around 1,000 g or 1 kg for *D. alata* cultivars and 500 g for *D. cayenensis rotundata* cultivars. The yield per hectare of these vitroplants is very high, reaching up to 67 tonnes/ha in some *D. alata* cultivars. Indirect weaning and soil amended with chicken droppings are best for producing large quantities of tubers for seed supply to farmers. In vitro cultivation makes it possible to obtain the seeds needed for high yam profitability and to safeguard all genetic resources by conserving them in the form of in vitro plants.

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