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Production of Yam Tubers Using Seed Tubers from Vitroplants Regenerated from Aerial Stems in 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 study is to produce large quantities of yam tubers in the field from seed tubers derived from vitroplants regenerated from aerial stem cuttings and farmers' tuber seeds. Seed tubers from ten yam cultivars, including five from *Dioscorea alata* (cDa083, cDa053, cDa115, cDa150 and cDa266) and five from *Dioscorea cayenensis-rotundata* (cDr015, cDr027, cDr150, cDr206 and cDr148) with distinct agro-morphological characteristics, were used as material. A completely randomized block design with 3 replications was used. In each block, all cultivars and seed types were represented. When put into cultivation, the tubers produced by the vitroplants all germinated and gave 100% healthy plants, compared with 86% to 100% healthy plants for the seed tubers produced by the farmers. Yields per hectare ranged from 10 to 25 tonnes for seed tubers produced by *in vitro* plants, and from 4 to 9 tons for seed tubers produced by farmers. Seed tubers from vitroplants can therefore be recommended to farmers as a solution to the problem of seed tuber unavailability.

Keywords

Yams, Vitroplants, Seed Tubers, Yields

1. Introduction

Yam (Dioscorea spp.) is one of the most important food crops in the tropics. It is consumed by thousands of people living in tropical and subtropical regions [1]. West and Central Africa constitute the so-called yam belt, accounting for 96% of the world's cultivated area and supplying 95% of global production, estimated at 71 million tonnes [2]. Nigeria is the world's leading producer, supplying 71.5% of global production [2]. With 7853083.92 tonnes, Côte d'Ivoire ranks 3rd worldwide after Ghana [3]. Not only do yams provide important carbohydrates, proteins and vitamins for human consumption, they are also significant sources of income [4]. They also play a decisive role in the socio-cultural relations of many traditional populations [5]. The main species cultivated in Côte d'Ivoire are *Dioscorea alata* and the *Dioscorea cayenensis-rotundata* complex [6]. The *Dioscorea alata* species (60% of production) is mainly destined for family consumption, while *Dioscorea cayenensis-rotundata* (40%) is preferably marketed [7] [8]. Annual yam production exceeds 200 kg per capita, making it Côte d'Ivoire's leading food crop [9]. Despite this, annual production is still insufficient to meet the food needs of Côte d'Ivoire's fast-growing population [10]. This is because seed requirements considerably reduce the quantity of tubers intended for consumption. In fact, more than 1/3 of the tubers produced for consumption are used as planting material. As a result, after the creation of new yam plantations, the unavailability of edible tubers has led to famine among farmers.

To solve this problem, several tuber-seed production techniques have been developed, notably vegetative propagation of yam by *in vitro* culture. This technique offers many advantages and has made it possible to find plant material other than tubers for seed production.

The aim of this study is to evaluate the tuber yield and health status of yams produced in the field from seed tubers derived from vitro plants regenerated from the aerial stems of several yam species.

2. Material and Methods

2.1. 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 [11].

2.2. Plant Material

The plant material consisted of seed tubers from vitroplants and seed tubers 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 *Di-*

oscorea 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.

2.3. Methods

2.3.1. Experimental Design

Trials were conducted in a randomized complete block design with three replicates. In each block, 10 tubers produced by plants grown from vitroplants of each cultivar and 10 traditional seeds of each cultivar (tubers produced by plants grown from germinated seed tubers) were grown on field mounds. Tubers were harvested 9 to 10 months after planting. Trials were carried out over two growing seasons.

2.3.2. Agronomic Yield Variables Evaluated

Agronomic yield variables were taken from each yam plant. Growth variables included plant height and number of tillers produced per plant, mean number of tubers per plant.

2.4. Data Analysis

A multivariate variance analysis (MVA) was performed with R software, version 4.1.2. The AMOVAs were supplemented by ANOVA tests when interactions were significant. 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 $P \leq 0.05$ [12].

3. Results and Discussion

3.1. Results

3.1.1. Sprouting Rate of Seed Tubers Produced by *in Vitro* Plants and by Plants Derived from the Sprouting of Tuber Fragments

Table 1 shows the germination rates of tuber-seeds from the *in vitro* plants and those of plants derived from germinating tuber fragments. For all cultivars, all tuber seeds from *in vitro* plants germinated. In contrast, the germination rate of

tuber-seeds from plants derived from tuber fragment germination varied from 86.66% to 100%. In *D. alata* cultivars, this rate varied from 90% to 100%, while in *D. cayenensis-rotundata* cultivars, it varied from 86.66% to 100%.

3.1.2. Anthracnose Infection Rate of Seedlings of Different Yam Varieties

For both yam species, *D. alata* and *D. cayenensis-rotundata*, seedlings derived from germination of seed tubers produced by the *in vitro* plants of all cultivars were free of symptoms of anthracnose caused by the plant-parasitic fungus *Colletotrichum gloeosporioides* (Table 2). The rate of healthy plants was 100%. However, plants of some cultivars of *D. cayenensis-rotundata* derived from sprouted seed tubers produced by plants obtained from tuber fragments were affected by anthracnose. Thus, the rate of healthy plants ranged from 86.66 to 100%. Figure 1 shows the appearance of leaves of anthracnose-affected [Figures 1(A)-(C) (1)] and disease-free [Figure 1(C) (2)] plants. Affected leaves show brown necrotic spots surrounded by yellow rings. *D. alata* cultivars were not affected even when their stems and those of diseased *D. cayenensis-rotundata* cultivars were carried by the same stake [Figure 1(C) (2)].

Table 1. Germination rate in % of seed tubers of different yam varieties.

Types of seeds	Yams varieties									
	cDa 083	cDa 053	cDa 115	cDa 150	cDa 266	cDr 015	cDr 027	cDr 150	cDr 206	cDr 148
Seed tubers of vitro plants	100	100	100	100	100	100	100	100	100	100
Seed tubers of plants from fragment of tubers	100	100	100	100	100	100	90	86.66	93.33	96.66

Cultuvars of *Dioscorea alata* (cDa083 or Florido; cDa053 or C18: improved variety, cDa115: Nza variety, cDa150: Nza variety, cDa266 or Kanga-nza). Cultivars *Dioscorea cayenensis-rotundata* (cDr015 or C20: improved variety, cDr027 or Krenglè; cDr150 or Krenglè doko tangbi; cDr206 or Kponan, cDr148 or Lokpa).

Table 2. Rate of healthy seedlings in % from germination of seed tubers of different yam varieties.

Types of plants	Yams varieties									
	cDa 083	cDa 053	cDa 115	cDa 150	cDa 266	cDr 015	cDr 027	cDr 150	cDr 206	cDr 148
Plants resulting from the germination of tuber seedlings of vitroplants	100	100	100	100	100	100	100	100	100	100
Plants obtained from the germination of tuber-seeds of plants obtained from tuber fragments	100	100	100	100	100	100	86.66	90	93.34	100

Cultuvars of *Dioscorea alata* (cDa083 or Florido; cDa053 or C18: improved variety, cDa115: Nza variety, cDa150: Nza variety, cDa266 or Kanga-nza). Cultivars *Dioscorea cayenensis-rotundata* (cDr015 or C20: improved variety, cDr027 or Krenglè; cDr150 or Krenglè doko tangbi; cDr206 or Kponan, cDr148 or Lokpa).

Figure 1. Leaf appearance of yam plants affected by anthracnose [A, B and C (1)] and disease-free [C (2)].

3.1.3. Yield of Yam Varieties in the Field According to the Type of Seed from Which the Plants Are Derived

On average for the cultivars of both yam species, there was no significant difference in the number of tubers produced per plant between seed tuber sprouted plants produced by *in vitro* plants and plants sprouted from tuber fragments (**Table 3**). However, the values of total tuber mass per plant (**Table 4**), average tuber mass (**Table 5**) and yield per hectare (**Table 6**) of the plants derived from sprouted seed tubers produced by the *in vitro* plants are significantly higher than those of the plants derived from sprouted tuber fragments. These mean data are similar to the results obtained in the overall analysis (**Table 7**). **Figure 2** shows the difference in size of tubers produced by plants germinated from seed tubers from *in vitro* plants and plants germinated from tuber fragments. Tubers produced by seed tuber sprouted plants from *in vitro* plants are generally larger and bigger than those produced by tuber fragment sprouted plants.

Table 3. Mean number of tubers produced by seedlings of yam varieties in the field according to the type of seed from which they are derived.

Yams cultivars	Seeds of vitroplants	Tuber seeds	Fisher Test	
			F	P
cDa053	1.00 ± 0.00[a]	1.00 ± 0.00[a]	2.41	0.2166
cDa083	1.40 ± 0.60[a]	1.3 ± 0.57[a]	0.99	0.4345
cDa115	1.60 ± 0.68[a]	1.45 ± 0.60[a]	1.34	0.2587
cDa150	1.75 ± 0.55[a]	1.60 ± 0.50[a]	0.76	0.4823
cDa266	1.20 ± 0.41[a]	1.25 ± 0.44[a]	1.18	0.3278
cDr015	1.15 ± 0.37[a]	1.05 ± 0.22[a]	2.47	0.0873
cDr027	1.60 ± 0.88[a]	1.40 ± 0.68[a]	2.39	0.0208
cDr148	1.30 ± 0.47[a]	1.20 ± 0.41[a]	0.96	0.6665
cDr150	1.15 ± 0.37[a]	1.00 ± 0.00[a]	2.09	0.3431
cDr206	1.00 ± 0.00[a]	1.00 ± 0.00[a]	0.21	0.8876

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 variety, cDa115: Nza variety, cDa150: Nza variety, cDa266 or Kanga-nza). Cultivars *Dioscorea cayenensis-rotundata* (cDr015 or C20: improved variety, cDr027 or Krenglè; cDr150 or Krenglè doko tangbi; cDr206 or Kponan, cDr148 or Lokpa).

Table 4. Total weights (in kg) of tubers produced by seedlings of yam varieties in the field according to the type of seed from which they were obtained.

Yams cultivars	Seeds of vitroplants	Tuber seeds	Fisher Test	
			F	P
cDa053	2.40 ± 0.82[a]	1.05 ± 0.37[b]	38.8	0.01
cDa083	1.33 ± 0.47[a]	0.59 ± 0.33[b]	33.7	<0.01
cDa115	2.25 ± 1.83[a]	0.94 ± 0.53[b]	77.8	<0.01
cDa150	3.19 ± 1.65[a]	1.24 ± 0.60[b]	89.7	<0.01
cDa266	1.61 ± 0.81[a]	1.05 ± 0.55[b]	15.5	0.04
cDr015	2.25 ± 0.74[a]	1.22 ± 0.40[b]	43.9	<0.01
cDr027	1.50 ± 0.62[a]	0.86 ± 0.57[b]	55.5	<0.01
cDr148	1.32 ± 0.47[a]	0.80 ± 0.46[b]	39.3	<0.01
cDr150	1.73 ± 0.58[a]	0.86 ± 0.41[b]	61.6	<0.01
cDr206	0.43 ± 0.22[a]	0.22 ± 0.14[a]	2.9	0.2915

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 variety, cDa115: Nza variety, cDa150: Nza variety, cDa266 or Kanga-nza). Cultivars *Dioscorea cayenensis-rotundata* (cDr015 or C20: improved variety, cDr027 or Krenglè; cDr150 or Krenglè doko tangbi; cDr206 or Kponan, cDr148 or Lokpa).

Table 5. Average tuber weights produced by yam varieties in the field according to the type of seed from which the plants were derived.

Yams cultivars	Seeds of vitroplants	Tuber seeds	Fisher Test	
			F	P
cDa053	2.40 ± 0.82[a]	1.05 ± 0.37[b]	22.17	<0.01
cDa083	1.05 ± 0.46[a]	0.48 ± 0.23[b]	31.14	<0.01
cDa115	1.38 ± 0.71[a]	0.71 ± 0.42[b]	37.29	<0.01
cDa150	2.03 ± 1.14[a]	0.83 ± 0.44[b]	46.78	<0.01
cDa266	1.31 ± 0.39[a]	0.85 ± 0.33[b]	42.47	<0.01
cDr015	2.03 ± 0.55[a]	1.18 ± 0.41[b]	51.29	<0.01
cDr027	1.00 ± 0.24[a]	0.62 ± 0.32[b]	29.71	<0.01
cDr148	1.03 ± 0.26[a]	0.65 ± 0.26[b]	31.31	<0.01
cDr150	1.54 ± 0.43[a]	0.88 ± 0.41[b]	26.94	<0.01
cDr206	0.43 ± 0.22[a]	0.22 ± 0.14[a]	1 87	0.211

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 variety, cDa115: Nza variety, cDa150: Nza variety, cDa266 or Kanga-nza). Cultivars *Dioscorea cayenensis-rotundata* (cDr015 or C20: improved variety, cDr027 or Krenglè; cDr150 or Krenglè doko tangbi; cDr206 or Kponan, cDr148 or Lokpa).

Table 6. Average yields per hectare (in tons) of yam varieties in the field by seed type.

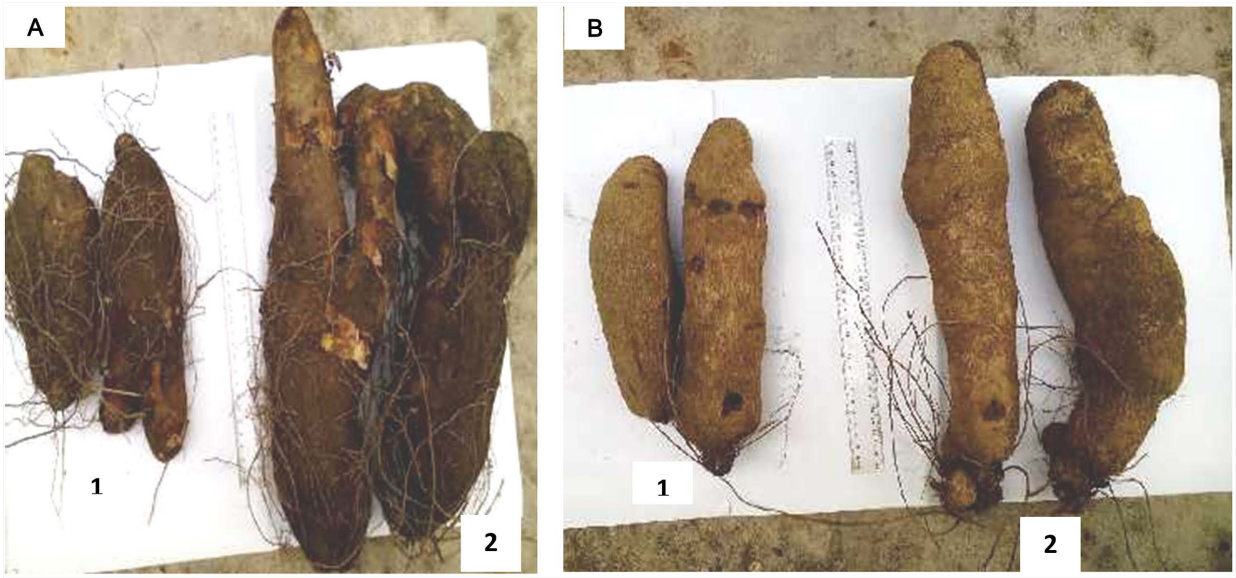
Yams cultivars	Seeds of vitroplants	Tuber seeds	Fisher Test	
			F	P
cDa053	19.20 ± 7.14[a]	8.40 ± 2.94[b]	118.23	<0.01
cDa083	10.64 ± 3.75[a]	4.72 ± 2.62[b]	84.16	<0.01
cDa115	18.00 ± 1.46[a]	7.78 ± 4.21[b]	123.34	<0.01
cDa150	25.52 ± 1.32[a]	9.88 ± 4.89[b]	145.21	<0.01
cDa266	12.88 ± 6.51[a]	8.36 ± 4.41[b]	47.39	<0.01
cDr015	18.16 ± 5.95[a]	9.76 ± 3.18[b]	99.19	<0.01
cDr027	11.96 ± 4.75[a]	6.88 ± 4.53[b]	76.71	<0.01
cDr148	10.56 ± 3.79[a]	6.40 ± 3.68[b]	43.16	<0.01
cDr150	13.84 ± 4.62[a]	7.04 ± 3.27[b]	67.14	<0.01
cDr206	3.44 ± 1.78[a]	1.76 ± 1.08[b]	14.11	<0.01

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 variety, cDa115: Nza variety, cDa150: Nza variety, cDa266 or Kanga-nza). Cultivars *Dioscorea cayenensis-rotundata* (cDr015 or C20: improved variety, cDr027 or Krenglè; cDr150 or Krenglè doko tangbi; cDr206 or Kponan, cDr148 or Lokpa).

Table 7. Yield variables values of yam seedlings in the field by seed type.

Yield variables	Seeds of vitroplants	Seed tubers	Fisher test	
			F	P
Mean number of tubers per plant	1.31 ± 00[a]	1.22 ± 00[a]	3.49	0.110
Total mass of tubers per plant (kg)	1.88 ± 1.05[a]	0.88 ± 0.33[b]	15.56	<0.01
Mean mass of a tuber (kg)	1.42 ± 0.46[a]	0.75 ± 0.27[b]	12.66	<0.01
Yield per hectare (tone)	14.71 ± 3.79[a]	7.06 ± 4.13[b]	15.56	<0.01

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



A: cultivar cDa053 of *D. alata*; B: cultivar cDr015 of *D. cayenensis-rotundata.*

Figure 2. Tubers of different sizes produced by seedlings of yam varieties from seed tubers (1) and from seed of *in vitro* plants (2).

3.1.4. Yield Variables of Germinated Seed Tubers Produced by *in Vitro* Plants of Different Yam Cultivars in the Field

The values of the yield variables of the germinated seed tubers produced by the *in vitro* plants are presented in Table 8. For all yield variables, there are very highly significant differences between cultivars. For *D. alata*, the mean number of tubers per plant ranged from 1.00 in cultivar cDa053 to 1.75 in cultivar cDa150. For *D. cayenensis-rotundata*, the mean number of tubers per plant ranged from 1.00 in cultivar cDr206 to 1.6 in cultivar cDr027. Cultivar cDa150 had the highest total tuber mass per plant (3.185 kg per plant) while cultivar cDr206 had the lowest tuber mass (0.43 kg per plant). The average mass of a tuber ranged from 1.05 kg to 2.40 kg in *D. alata* and from 0.43 kg to 2.03 kg in *D. cayenensis-rotundata*. The highest average mass, 2.40 kg and the lowest 0.43 kg were obtained in cultivars cDa053 and cDr206 respectively. Yield per hectare ranged from 25.52 tons/hectare for cultivar cDa150 to 3.44 tons/hectare for cultivar cDr206.

Table 8. Comparison of plant yield variables of different yam cultivars in the field.

Yams cultivars		Mean number of tubers per plant	Total mass of tubers per plant (kg)	Mean mass of a tuber (kg)	Yield per hectare (tons)
cDa053		1.00^{c}	2.39^{b}	2.40^{a}	19.20^{b}
cDa083		1.40^{abc}	1.33^{d}	1.05^{b}	10.64^{d}
cDa115		1.60^{ab}	2.25^{bc}	1.38^{a}	18.00^{bc}
cDa150		1.75^{a}	3.18^{a}	2.03^{a}	25.52^{a}
cDa266		1.20^{bc}	1.61^{bcd}	1.31^{b}	12.88^{bcd}
cDr015		1.15^{bc}	2.27^{bc}	2.03^{a}	18.16^{bc}
cDr027		1.60^{ab}	1.49^{cd}	1.00^{c}	12.06^{cd}
cDr148		1.30^{bc}	1.31^{d}	1.03^{b}	10.56^{d}
cDr150		1.15^{bc}	1.73^{bcd}	1.54^{b}	13.84^{bcd}
cDr206		1.00^{c}	0.43^{d}	0.43^{c}	3.44^{e}
Fisher Test	F	5.43	12.47	19.97	12.47
	P	<0.01	<0.01	<0.01	<0.01

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

3.2. Discussion

The aim of this study was to produce yam tubers in the field using seed tubers from vitroplants regenerated from aerial stem. The results showed that the seeds produced by plants germinated from *in vitro* plant seed tubers outperformed those produced by plants germinated from seed tubers. In fact, the germination test showed that the seeds produced by plants germinated from tuber-seed *in vitro* plants all germinated. Analyses also showed that these seed tuber plants from vitroplants were healthy, as diseases caused by fungi such as anthracnose and rots, nematodes, viruses (*Yam Mosaic Virus* (YMV) and Internal Brown Spot (IBS) were not observed on these plants. The absence of disease in seed tubers grown from *in vitro* plants could explain the 100% germination rate obtained with these seed tubers. The elimination of these diseases is thought to be due to the cultivars being grown *in vitro*. The results obtained are similar to those of Ng [13], who obtained 300 clones free of yam mosaic virus through *in vitro* culture. The same applies to the results of Saleil [14], who succeeded in ridding affected plants of YMV by *in vitro* cultivation of the apexes of contaminated nodal cuttings.

Regarding the average number of tubers per plant, the results showed that there was no difference between the number of tubers produced by the two types of seed, whatever the yam cultivar. As for the average mass of a tuber per plant, the results showed that the average masses of seed tubers from vitroplants were

higher than those from germinated seed tubers, with the exception of cultuvars cDr206, whose average masses were statistically identical for both types of seed. Similarly, tables showing the average tuber mass per plant and the yield of fresh yam tubers per hectare showed statistically different results depending on the type of seed used. The average tuber mass per plant and yield of fresh yam tubers for plants germinated from tuber seeds produced by *in vitro* plants was more than double that of seeds produced from plants germinated from tuber seeds. These results could be explained by the fact that the disinfection achieved by *in vitro* cultivation improved the performance and vigour of the seeds produced by the vitroplants, and consequently increased production. Similar results were observed by Yao [15]. Indeed, after treating yam seedlings with aqueous extracts plus adjuvant, the author recorded the best yields. This adjuvant improved the efficacy of the aqueous extracts against yam anthracnose. As the yam plants do not show any symptoms of anthracnose, biological functions such as absorption of water and mineral elements by the roots, photosynthesis, and transport and storage of the products of photosynthesis, can be carried out correctly. The nutrient reserves regularly produced by these yam plants during photosynthesis would gradually accumulate in the tubers, boosting production.

4. Conclusion

The work carried out has made it possible to produce yam tubers in the field from seed tubers derived from votroplants of two yam species. The production of seed tubers in the field from cultivars of the two yam species, *D. alata* and *D. cayenensis-rotundata*, has been improved by the use of seeds preserved in the form of vitroplants. Tubers produced by *in vitro* plants are of very high quality, not only because they are healthy and resistant to the main yam diseases, but also because they produce highly productive plants with a very good yield per hectare, unlike tubers produced by plants grown directly from germinating seed tubers. Thus, the production of seed tubers by *in vitro* cultivation is far better than that achieved by the traditional method of growing plants directly from the germination of tuber fragments. *In vitro* cultivation makes it possible to obtain the healthy seeds needed for high yam profitability and to safeguard all genetic resources by conserving them in the form of *in vitro* plants. It is therefore possible to solve the thorny problem of seed unavailability and satisfy farmers by supplying them with thousands of tons of tubers.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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