

## Effect of local substrates on the development of microtubers of yam (*Dioscorea rotundata* L.) varieties using the SAH technique

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## Abstract

The inadequacy of the quantity and quality of production equipment is a major concern for yam production in Benin. Likewise, the new technique of mass production of microtubers is limited by problems related to the supply of the modern substrate. The objective of this study is to identify a local substrate based on plant residues with the potential of those imported (modern substrates) to limit their use in SAH. A control consisting of modern substrate TS3 (SM) and two substrates containing different local residues (100% carbonized peanut shell and 100% carbonized sawdust) were tested on varieties Kouna, Bobo, TDr 9519177, and TDr 8902665. A completely random two-factor bulk experimental design repeated three times was used. The plants were followed through the collection of growth data such as the plants' number, plant height, the number of branches, and the length and width, and then at the harvest, data including the number of microtubers, microtubers diameter, and weight were collected. The results obtained show that the mean of all the parameters considered for the growth and the post-harvest phase are higher with the SM substrate than those of the CA and SB substrates. In addition, discriminating parameters such as the number of microtubers, diameter of microtubers, and weight of microtubers had higher mean values (3.5; 5.37 mm; 037 g) in SB than in CA (2.08; 3.07 mm; 0.19 g), respectively. Plants seeded in the SB substrate showed significant growth gains ranging from 45 to 99% in height and from 35 to 97% in leaf width. Substrate SB, therefore, represents a good medium for the growth and production of microtubers that can replace modern substrate TS3 (SM). Nevertheless, physicochemical characterization and enrichment of the sawdust substrate should be considered to optimize the yield of yam microtubers.

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**Keywords:** Yams, Local Substrates, Microtubers, SAH Technique, Benin

## Introduction

The yam (*Dioscoreo spp*) of the family *Dioscoreoaceae*, is a tuber plant of great food, economic and socio-cultural importance in the tropical world and especially in West Africa (Ogunlade *et al.*, 2011; Amoo *et al.*, 2014). Among root and tuber plants, yam, with a production of 71 million tons in 2017, is the basis for the diet of more than 500 million people in some tropical countries in Africa, the Caribbean, and Latin America (Onyeka *et al.*, 2006, FAOSTAT, 2022).

In Benin, the annual yam production over the last ten years is 2730000 tons (DSA, 2017). In the 2020-2021 crop year, Benin's production was 3150248 tons, or 5% of world production (DSA and APRM, 2021), ranking it fourth among yam-producing countries after Nigeria (70%), Côte

d'Ivoire (9%), and Ghana (7%). Yam is therefore considered to be one of the strategic products for food security in Benin, where the departments favorable to its production are the Collines, Borgou, Donga, Atacora, Zou, and Plateau (Adifon *et al.*, 2019). Vernier and Dansi (2006) estimate yam cultivars in Benin at 313, grouped into 26 morphotypes.

In the context of the revitalization of the yam sector, the availability of seed is the first of the challenges, since on average 25 to 50% of the proportion of yam harvests are converted into the seed (Dibi *et al.*, 2014). Thus, several studies have been carried out to find an appropriate technique for the mass production of seeds. In traditional cultivation, propagation is by vegetative means; the plant is born from a tuber fragment known as a cut, planted on planting (Ile *et al.*, 2006). Nevertheless, the multiplication coefficient from this method is very low. In 1990, a technique was developed called the miniset technique, which uses mini-tuber fragments of 20 to 50 g. However, the high cost and low rate of return do not solve the producers' difficulties. In the current era, the work has led to much more efficient and cost-effective techniques, including producing yam seeds by in vitro cultivation with various vegetative propagation modalities. In vitro, vegetative propagation has a priori numerous advantages (unequal multiplication rate), allowing very large-scale production of material occupying little space and reducing the cost per plant produced (Dibi *et al.*, 2016). Even more recently, IITA has developed one of the above-ground cultivation techniques, the semi-autotrophic hydroponic cultivation practiced in Nigeria, which produces 70% of the world's production. This culture technique uses virus-free tissue culture seedlings as planting material in plastics containing culture media called an imported substrate. Semi-autotrophic hydroponic technology (SAH) is efficient for the clean production of seedlings and mini tubers and is easy to make (Olugboyega *et al.*, 2019). The importance of this study is consistent with the need to further investigate the adaptation of certain plant residues to organic substrates to reduce not only the purchase costs of imported substrates but also the inconvenience associated with their supply difficulties. In research for the determination of substrates based on plant residues, work was carried out on composting plantain bananas (Atsin *et al.*, 2019, Molongo *et al.* 2021). Not only has work been done by Coulibaly *et al.* (2021) on the hydroponic cultivation of tomatoes with substrates based on coconut fiber, soy compost, and peanut shells but also by Kouame *et al.* (2023) for nursery cultivation of *Terminalia Ivorensis* from forestry and agricultural waste. In yam production, the aim is to optimize the production of yam microtubers by developing local substrates based on plant residues to limit the use of imported substrates. This research and development support yam producers by providing healthy and sufficient seeds to boost production and help

address the food insecurity of the population. The present study assessed the influence of plant residue substrates on the development of cuttings of different yam cultivars using the SAH technique. To carry out this work, specific objectives are set:

- Evaluate the effect of each of the local substrates on the development of cuttings of different yam cultivars.
- Identifying among the local substrates, the one that gives more yield of microtubers.
- Assess the ability of the various improved and local cultivars studied to grow and develop with SAH technology

### **Plant material**

The Laboratory of Genetics, Biotechnologies and Applied Botany (GeBBA) in which the work was carried out is located in the National Higher School of Biosciences and Applied Biotechnologies of Dassa-Zoumè in the Center of Benin.

The plant material consists of 04 cultivars of yam (*Dioscorea rotundata*). These are the local varieties Kouna and Bobo, and then the improved accessions TDr 9519177 and TDr 8902665 were selected based on their agro-morphological performance, nutrient values, and growth rates. These cultivars were collected as vines in the greenhouse and growing room on the feet already under development in semi-autotrophic hydroponic culture.

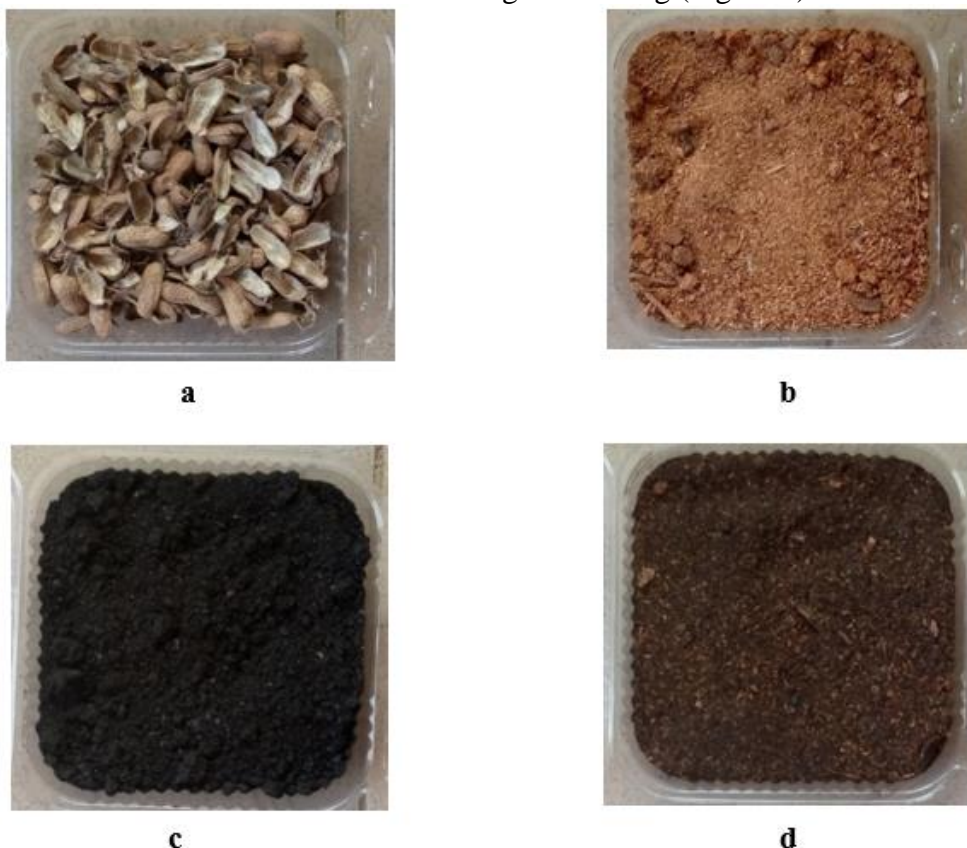
### **Methods**

#### **Preparation and Composition of Plant Residue Substrates**

Substrates are solid media that support the plant, from which it will draw some of the nutrients and moisture it needs to make its food. Our choice of substrate has been organic substrates since they are of natural origin and come from certain plant residues such as peanut shells; sawdust or wood chips. Thus, the two plant residues selected as material for the preparation of the plant residue substrates: sawdust and peanut shell were obtained respectively in a sawmill and a peanut grainage plant.

The groundnut shells were crushed in the lab with a pestle mortar to produce a powder that resembles sawdust. Once the manufacturing equipment was ready, a wood fire was made outside the laboratory for the carbonization of the residues. This carbonization is carried out to sterilize the residues based on their origin, and then to make available the nutrients present in the medium once the substrates have been obtained. A metal barrel, vertically cut in half, was used as an incinerator for the carbonization of the two residues. The sawdust was charred for one hour on an open fire for each charred amount due to the nature of the material. The carbonization

of the peanut powder lasted only 30 minutes in the open air. After each carbonization, a drying of approximately one hour and thirty minutes is carried out for each substrate to ensure good cooling (Figure 1).



**Figure 1:** Different local residues before and after carbonization  
(a: residues peanut shells; b: residues sawdust; c: carbonized peanut shells; d: carbonized sawdust)

### **Preparation and composition of culture media**

The media used for transplanting are the substrates previously prepared from plant residues: sawdust (sawdust substrate), and groundnut shells (groundnut substrate). Next, the modern substrate (TS3), which is a commercially available substrate already adopted for HSA cultivation to assess the effect of plant residue substrates on the development of yam cuttings. In each HSA box (plastic), 500 ml of each type of substrate (SB; CA, and SM) are distributed and then moistened with 250 ml of fertilizing solution. The fertilizing solution is prepared from  $\frac{1}{2}$  tablespoon of coarse miracle (a set of mineral salts) in four liters (4 L) of well-homogenized tap water and left for handling the next day.

### Preparation for handling

Each time the table is handled, it is dusted with table paper and then carefully cleaned with 70°C alcohol-soaked table paper. The blades, scalpel, and scissors are also cleaned with 70°C alcohol. The mesh bags are then washed with tap water to collect the explants and the 10°C bleach solution is prepared.

### Transferring of explants

The explants used consist of yam vines from SAH for the varieties TDr 9519177 and TDr 8902665, and the greenhouse for the local varieties Kouna and Bobo. After the explants have been collected, the mesh bags are labeled with adhesive paper bearing the name of the variety. The mesh bags are washed with tap water and soaked in a 10% bleach solution for disinfection and then a final rinsing with tap water is done. On aluminum foil that has been cleaned with alcohol at 70°C, the micro-cuttings are cut according to the position of the bud and at the level of the leaves, using a scalpel with a blade. Each micro-cut comprises a knot. The explant thus prepared is then seeded vertically in SAH dishes, each containing 500 mg of substrate in the proportion of 16 microcuttings per dish. The SAH boxes are then closed and properly labeled.

### Experimental design

The SAH boxes thus closed were arranged in three blocks in the culture room with a temperature of 25° C., a photoperiod of 12 hours under a light intensity of 3000 lux, and a relative humidity maintained at 80%. The trial was conducted using a randomized full-block design with two factors including three substrate types and four varieties. A total of 192 plants were used, with 64 plants per block repeated 3 times.

### Parameters evaluated

Observations and measurements began at the 3<sup>rd</sup> week of transplanting and continued until the 90<sup>th</sup> day of transplanting, then in 3 months at 2-week intervals. Growth parameters such as seedling height or creeper length, leaf length, leaf width, and branching number were recorded. At 90<sup>th</sup> days after harvest, the number of microtubers, the diameter of the microtubers, and the fresh weight of the microtubers were evaluated (Table 1).

**Table 1:** Considered parameters and methods of measurement

Characters	Codes	Measurement methods
Number of plants	NP	Count the number of plants on the substrates
Plant height	PH	Main axis length (line)
Branching Number	BN	Count the number of branching to the main leaf part
Leaf Length	LL	Distance between leaf end and leaf base (main rib)



Leaf width	LW	Distance between ends opposite the length
Number of microtubers	NmicroT	Count the number of microtubers inside the substrates
Diameter of microtuber	DmicroT	Measure the circumference of each microtuber
Weight of microtubers	WmicroT	Weigh the microtubers

## Statistical analyzes

Data were subjected to a descriptive analysis using Excel 2016 software. The principal component analysis (PCA) was performed using Minitab 16 software. Graphs showing the evolution of the different aspects of the development of each variety as a function of time were produced with the Graph Pad Prism 9.5.1 (733) software.

## Results

### Variability of measured substrate parameters

The descriptive statistics of the recorded parameters from each of the four yam varieties are compiled in Table 2. This indicates that the means of all the parameters of the SM medium are higher than those of the AC and SB media. In addition, the parameters related to the architecture of the microtubers (NmicroT, DmicroT, WmicroT) have respectively larger values (3.5 mm; 5.37 mm; 037 g) in the SB medium than the values (2.08 mm; 3.07 mm; 0.19 g) obtained in the CA medium (Table 2).

Apart from these parameters where a large variation is observed around the mean; similar observations are detected with NP for the three media CA, SB, and SM with respective means of  $7.02 \pm 5.87$ ;  $7.9 \pm 5.01$  and  $13.47 \pm 3.53$ . Also, a large distribution around the mean is observed with the plant height (PH) on the SM media with a mean value of  $6.64 \pm 4.02$  (Table 2).

**Table 2:** Descriptive statistic of trait

Traits	CA				SB				SM			
	Min	Max	Mean	Std	Min	Max	Mean	Std	Min	Max	Mean	Std
NP	0	16	7.02	5.87	0	16	7.9	5.01	3	16	13.47	3.53
BN	0	2	0.11	0.37	0	0	0	0	0	9	1.13	2.05
PH	1	4.6	2.98	0.81	2	4.7	3.31	0.86	1.5	24.7	6.64	4.02
LL	2	4.8	3.47	0.78	2	4.1	3.3	0.53	2.2	7	4.18	0.98
LW	3	5.5	3.98	0.77	2.5	4.6	3.71	0.68	2.5	5.7	4.26	0.96
NmicroT	0	8	2.08	2.81	0	7	3.5	2.5	0	13	6.83	4.65
DmicroT	0	7.05	3.07	3.35	0	8.94	5.37	3.4	0	12.7	7.6	3.86
WmicroT	0	0.76	0.19	0.28	0	0.81	0.35	0.27	0	4.47	1.96	1.8

Min: minimum; Max: maximum; Std = standard deviation, NP: Number of plants; BN: Branching number, PH: Plant height; LL: Leaf length; LW: leaf wide; NmicroT: number of microtubers; DmicroT: diameter of microtuber; WmicroT: weight of microtubers

**Table 3:** Distribution of traits on the principal component axe

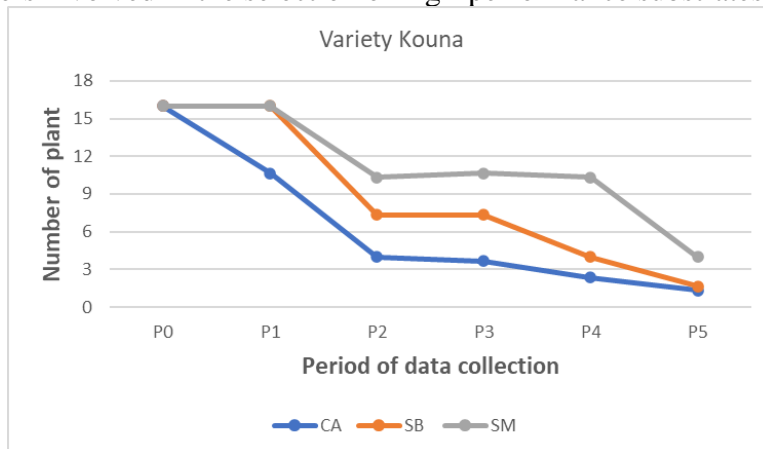
Variables	AC Medium			SB medium			SM Medium		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
NP	0.426	-0.269	0.395	0.324	0.519	-0.591	0.368	0.430	0.289
PH	0.273	0.343	-0.756	-0.369	-0.258	-0.629	0.408	-0.154	0.548
LL	0.108	0.631	0.232	-0.413	0.073	0.293	0.303	-0.457	-0.589
LW	0.109	0.602	0.397	-0.283	0.755	0.072	0.063	-0.712	0.422
NmicroT	0.502	-0.017	0.082	0.402	0.185	0.384	0.435	0.257	-0.021
DmicroT	0.494	0.001	-0.219	0.411	-0.235	0.005	0.450	0.036	-0.289
WmicroT	0.473	-0.221	0.077	0.421	0.006	-0.131	0.459	-0.086	-0.085
Eigenvalue	3.9401	2.2965	0.7634	5.6033	0.9634	0.4333	4.6730	1.7198	0.6071
Proportion	0.563	0.328	0.109	0.800	0.138	0.062	0.668	0.246	0.087
Cumulative	0.563	0.891	1.000	0.800	0.938	1.000	0.668	0.913	1.000

NP: Number of plants, PH: Plant height; LL: Leaf length; LW: leaf wide; NmicroT: number of microtubers; DmicroT: diameter of microtuber; WmicroT: weight of microtubers

The Principal Component Analysis carried out allowed the grouping of the modalities of the traits according to the first two axes which represent 89.1%, and 91.3% of the total variability of the respective media CA, and SM. These axes with eigenvalues greater than or equal to 1 allow us to better describe the distribution of the variables on each axis whatever the medium. Furthermore, with the medium SB, only the first axis with 80% of the total distribution of traits shows an eigenvalue greater than 1.

Thus, the number of plants, the number of microtubers, the diameter of the microtubers, and the weight of the microtubers are well correlated on the first axis of the main component. As for the second axis, the length of the leaves and the width of the leaves are better represented therein.

Consequently, the parameters relating to the subterranean development, which constitutes the seed sought in the context of yam production, such as the number of microtubers, the diameter of the microtubers, and the weight of the microtubers, are the most discriminating parameters involved in the selection of high-performance substrates.





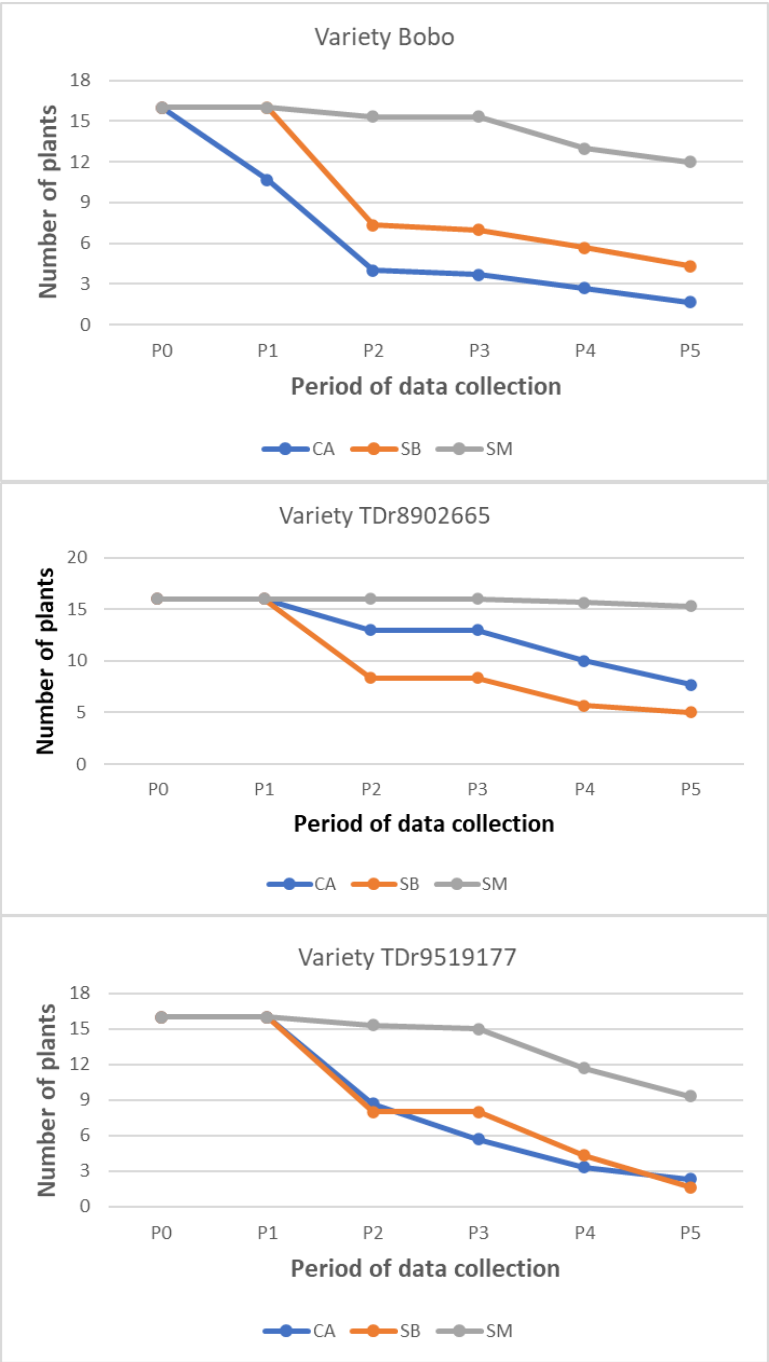
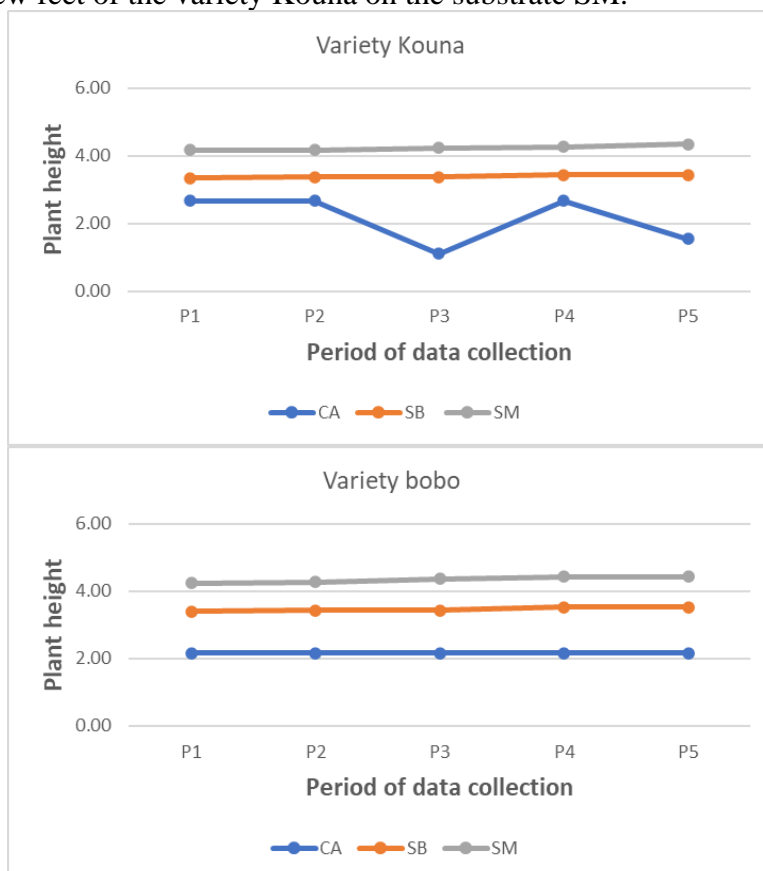


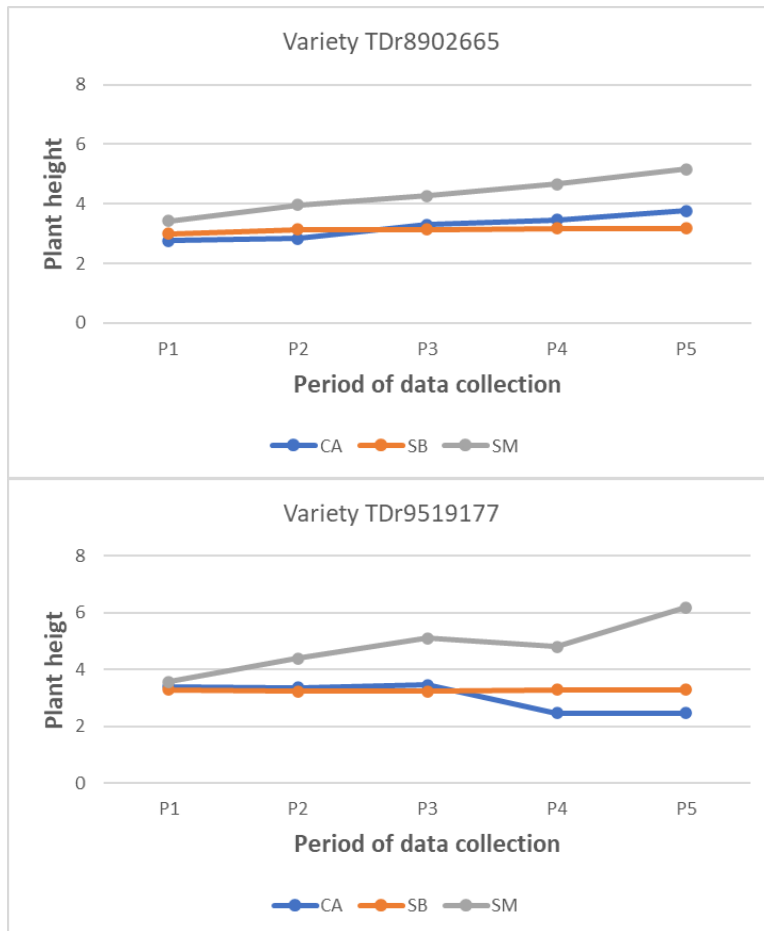
Figure 2: Change in the number of plants

Growth and development of yam varieties

Figure 2 shows the progress of the number of plants of the different varieties from the first period (P1) to the last period (P5) of data collection.

The results indicated a progressive drop in the number of plants transplanted on the three substrates. From the first period of data collection (P1), a large variation in the number of plants, i.e. 16 to 11 with the Bobo variety on the CA substrate and 16 to 10 with the Kouna variety on the CA substrate, was observed. However, no change in the number of plants was recorded for the TDr 8902665 and TDr 9519177 varieties on the three substrates. With the SB substrate, the variations were recorded from the second data input (P2) for all yam varieties and from the third data input (P3) with the SM substrate. Seedling losses were much greater on SB than on CA throughout the test (90<sup>th</sup> day). The varieties remaining in large numbers on the various substrates were the varieties TDr 8902665 and TDr 9519177 and then a few feet of the variety Kouna on the substrate SM.



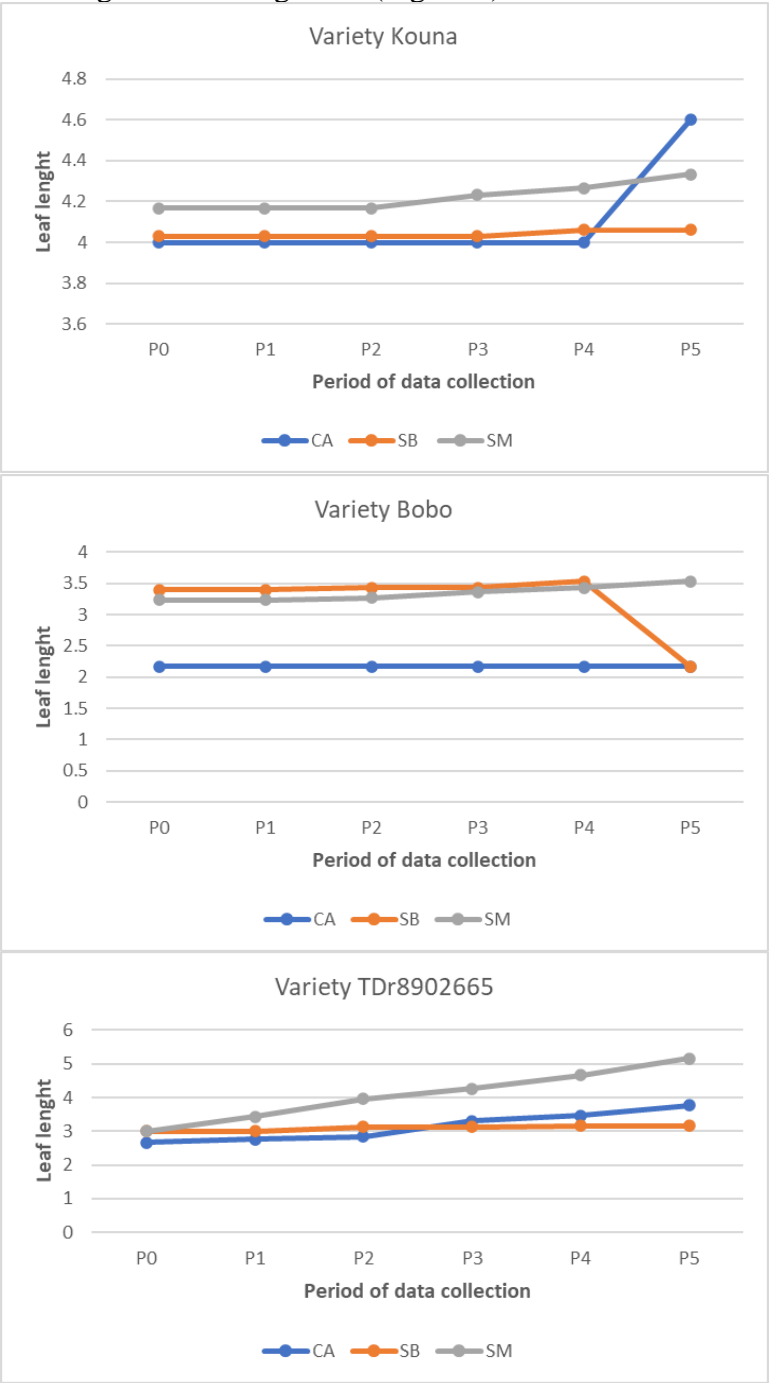


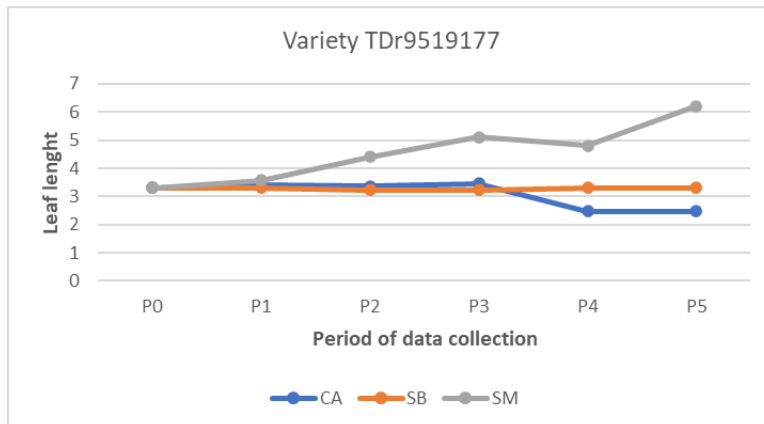
**Figure 3:** Growth in height or length of vines

The analysis in Figure 3 showed a gradual change in plant height over time for all varieties. The highest height growth was induced by the SM substrate for all of the transplanted varieties. Substrate SB exhibited the lowest values, out of the five data points. It was noted that the height of the vines on the SM substrate is greater in the varieties TDr 8902665 (3.5 cm to 24 cm) and TDr 9519177 (4 cm to 12 cm) compared with that of the Kouna and Bobo varieties.

The development of the leaves has evolved according to the variety, but also the type of substrate. At the beginning of the sowing (P0), the varieties with the largest leaf lengths were Bobo and Kouna. Nevertheless, from the first period of data collection, it was at the level of the varieties TDr 8902665 and TDr 9519177 that the onset of leaf growth was noted with the SM and CA substrates. With the variety TDr 8902665, this development was done progressively on the CA and SM substrates. At the last period of data

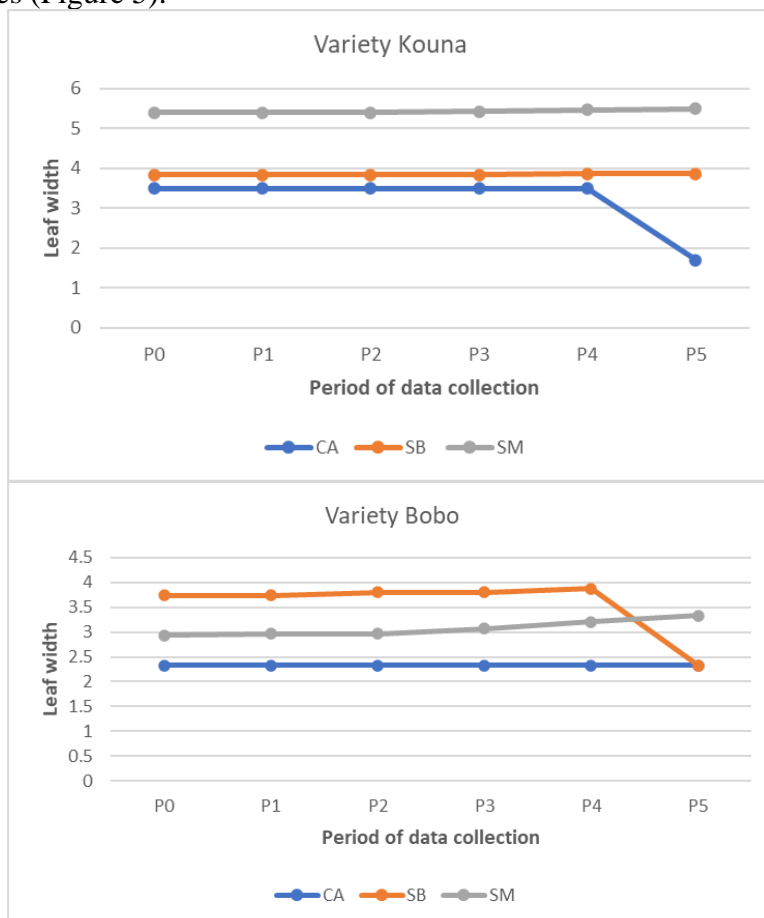
collection (P5), for all varieties combined, it was the substrate SM and CA that promoted significant leaf growth (Figure 4).

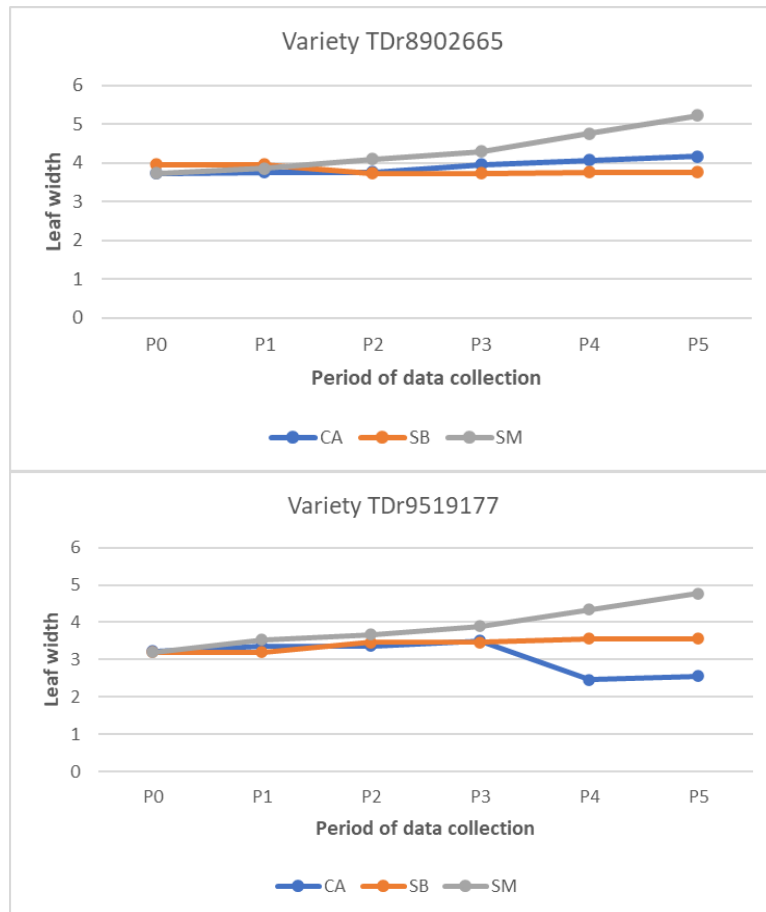




**Figure 4:** Leaf Length

As reported for leaf length, leaf width development was only truly discriminating between TDr 8902665 and TDr 9519177 with SM and CA substrates (Figure 5).

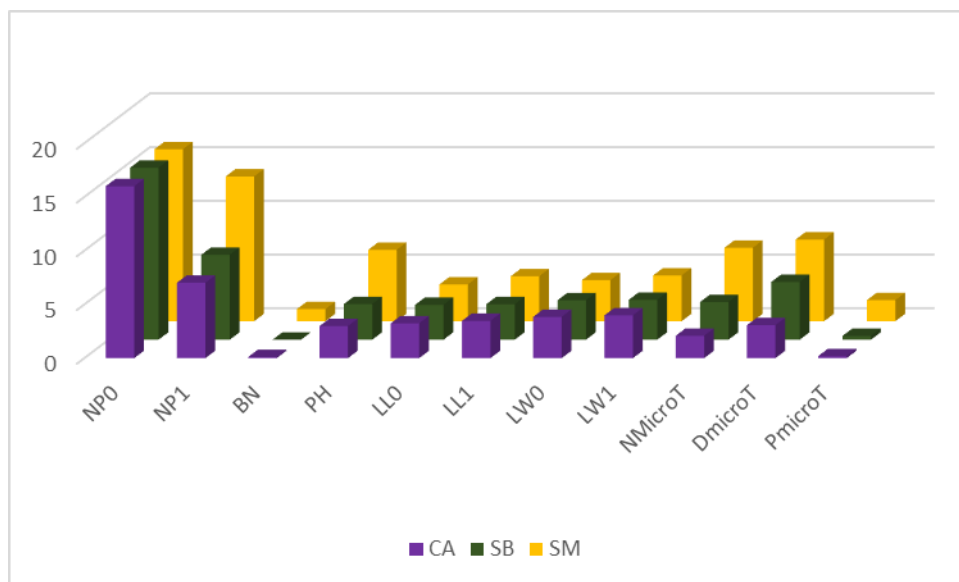




**Figure 5:** Leaf Width

### Effects of substrates on recorded traits

The SM substrate (TS3), composed of 100% compost, induced the best performance for all parameters (Figure 6). Of the two local substrates made up of 100% peanut shells and 100% sawdust, respectively, it is the SB substrate that has a better performance of the discriminating parameters such as the number, the diameter and the weight of microtubers.



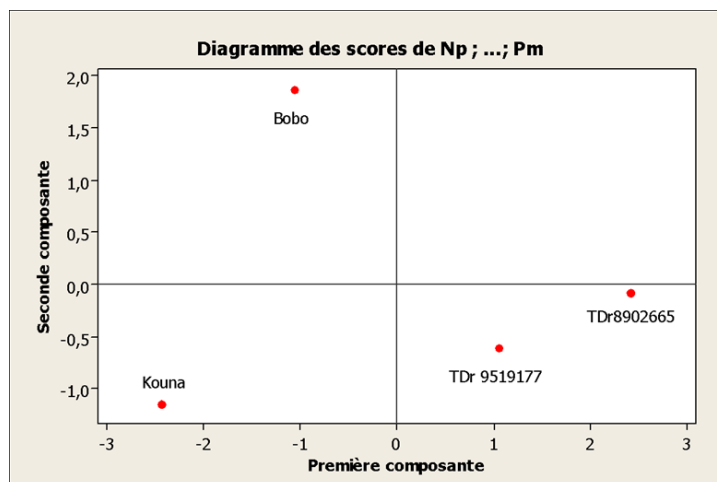
**Figure 6:** Effects of substrates on measured parameters

### Suitability of varieties for SAH technology

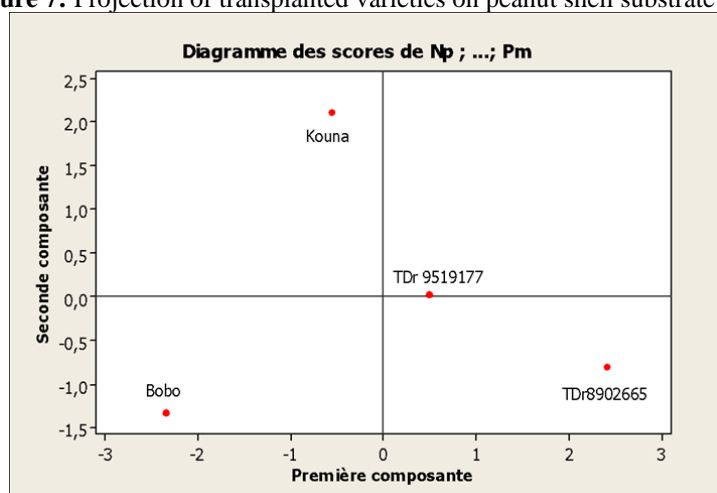
For each of the substrates, the projection of the yam varieties in the factorial axis defined by the two main components shows the ability of these varieties to grow and to develop not only as a function of the substrate but also with the SAH technique.

In the medium containing peanut (CA) shell, the varieties TDr8902665 and TDr 9519177 showed a large number of plants and microtubers with a large diameter and a high weight; in contrast to the Bobo and Kouna varieties, which are local varieties never involved in the SAH technique and which showed very low yields (Figure 7). For the sawdust (SB) culture medium, the TDr8902665 and Bobo varieties are those with a large number of plants, large diameter microtubers, and high weight with shorter vines and leaves, unlike the Kouna variety (Figure 8). Finally, with the modern substrate, the varieties TDr8902665 and TDr 9519177 are those showing a large number of plants with long vines and leaves and also many microtubers with large diameters and high weight, unlike the varieties Bobo and Kouna. The Bobo variety is then the one that has very long and also wide leaves, unlike the Kouna varieties (Figure 9).

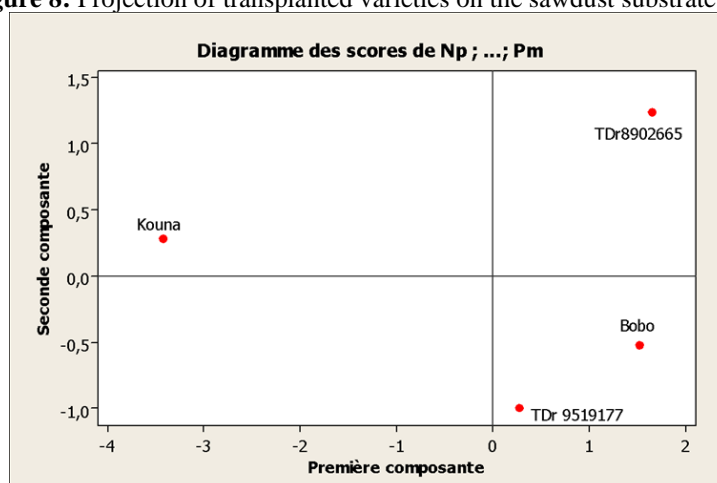




**Figure 7:** Projection of transplanted varieties on peanut shell substrate (CA)



**Figure 8:** Projection of transplanted varieties on the sawdust substrate (SB)



**Figure 9:** Projection of transplanted varieties on modern substrate (SM)

In short, whatever the substrate used, the varieties TDr 8902665 and TDr 9519177, which are varieties obtained by the SAH technique, are more suitable for developing in SAH culture, followed by the Bobo variety, which is more suitable than the Kouna variety; both of the local varieties are newly involved in HSA. Moreover, with modern substrate (SM), the yields obtained for microtubers are higher than those for SB substrate and the lowest yield is obtained with peanut shell substrate (CA). Overall results indicate that of the two local substrates used, SB has the best microtuber yields (number, diameter, and weight of microtubers), while CA produces better aerial vegetative development (leaf length and width).

## Discussion

The present study assessed the influence of plant residue substrates on yam cutting development and identified the one most closely related to the modern substrate.

The substrates used influenced the growth and development of both the aerial and root systems of yam cuttings. Compared with the control, with 93.8% of the results that can be explained, an abundance of plant numbers is noted with the substrate SB. This abundance of plant numbers is accompanied by shorter vines and leaves, large diameter, and high-weight microtubers. This could be justified by the water retention capacity of sawdust, its  $\text{pH} < 7$ , and its oxidizing power, which contributed to the development of the radicle; a prerequisite for emergence (Steward *et al.*, 1997). Therefore, this low level of seedlings with shorter vines and leaves on the substrate may be justified by the high rate of decomposition and the lack of nitrogen in sawdust. According to Aho and Kossou (1997), nutrients whose nitrogen stimulates vine vegetation accelerate the formation and growth of plant vegetative organs. These results are contrary to those of Kouame *et al.*, (2023), which obtained a germination rate of *Terminalia ivorensis* plants greater than 60% with a sawdust-based substrate. Still referring to the control substrate (SM), 89.1% of the results obtained with the substrate CA could be explained. This substrate shows a performance in plant production with very long vines, leaves as long but with fewer large diameters, and high-weight microtubers. This effect can be attributed to the nature of the substrate and to certain physical and physicochemical characteristics that make up the substrate (Coulibaly *et al.*, 2021; Molongo *et al.*, 2021). Due to the rapid depletion of organic and nutrient content of this substrate (CA), the number of microtubers obtained was not large. These results are consistent with those of Boufares (2012), who noted that during development, growth vigor is not necessarily varietal as is commonly recognized, but may also result from certain environmental conditions. In terms of selection, these data indicate that SB substrate having the desirable

characteristics as indicated above will easily improve the production of SAH microtubers. It emerges from these results that the substrate based on plant residues best suited for the production of microtubers would be the substrate SB.

The various results show that the most efficient varieties are the TDr8920665 and TDr9519177 varieties. Whatever the type of substrate, the varieties TDr8920665 and TDr9519177 are the ones with a large number of plants, and many microtubers with large diameters and high weight, unlike the Bobo and Kouna varieties. The performance of these two TDr varieties could be justified by their improved high-yield nature and already adapted to the SAH culture, unlike the Bobo and Kouna varieties which are local varieties newly introduced to SAH.

## Conclusion

The overall objective of this study is to optimize the production of yam microtubers through the development of local substrates based on plant residues to reduce the use of imported substrates. The results obtained showed that all organic substrates based on plant residues induce yam cuttings to grow, but the growth does not yet reach the growth performance induced by the SM substrate. SB was the most significant of the local substrates with an average number of 3.5 microtubers, an average diameter of 5.37 mm and an average fresh weight of 0.35 g compared to an average number of 2.08 microtubers, an average diameter of 3.07 mm, and an average fresh weight of 0.19 g at the CA substrate at harvest. This study thus proved that the SB substrate constitutes a good substitute substrate for the imported substrate for the production of SAH microtubers. To provide a more efficient sawdust-based substrate, it would be sensible to characterize this residue from the physical point of view (total porosity, aeration porosity, retention porosity, apparent density) and the chemical point of view (pH, electronic conductivity, organic matter, nitrogen, potassium).

**Authorship:** Adjatin Arlette, Dedavi Sidoine, Ogou Honorine, Tossa Crispus, Bossa Yasmine and Dansi Alexandre contributed to the study's conceptualization and designed the experiments. Adjatin Arlette and Bonou-Gbo Zaki contributed to the statistical analysis. Adjatin Arlette, Dedavi Sidoine and Dansi Alexandre wrote the manuscript. Odjo Tiburce, Fakorede Jeannette and Dansi Alexandre supervised the entire study. Bonou-Gbo Zaki, Bossa Yasmine, Odjo Tiburce, Fakorede Jeannette and Dansi Alexandre reviewed and edited the manuscript. All authors contributed to the manuscript.

**Conflict of Interest:** The authors reported no conflict of interest.

**Data Availability:** All data are included in the content of the paper.

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