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GROWTH RESPONSE OF PLANTS DERIVED FROM PATHOGEN-FREE BANANA FRAGMENTS TO DIFFERENT SUBSTRATES

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ABSTRACT

Production of vivoplants of banana and plantain is an important step in the use in the field of healthy planting material, free of bacterial or viral infection. The objective of this study was to assess the recovery and the subsequent seedlings growth from banana fragments to different substrates for rapid multiplication of banana suckers in Benin. An experiment was set up following a two-factor split plot arrangement with three repetitions. The first factor was the substrate at three levels (White sawdust, Coconut peat + sand and White sawdust + cassava effluent); and the second was the cultivar free from bacterial and viral infections, with four levels (Aloga, Planta, Sotoumon and Gunkoékoé), totally 12 treatments. From the results, the appearance of the buds depended on the nature of the substrate and the type of cultivar. Coco peat + sand and sawdust + cassava effluent were significantly different ($P<0.001$) in influencing the number of leaves and roots, leaf area, plant height, diameter at the collar of the seedlings and the number of young seedlings per banana explant. The highest number of leaves and roots, and young seedlings; and greatest plant height and diameter were obtained with coco peat + sand and white sawdust + cassava effluent, at the collar of the seedlings. From this study, it is clear that coconut peat + sand is the best substrate for the rapid multiplication of banana plantlets from the fragments method.

Key Words: Cassava effluent, coconut peat, white sawdust

RÉSUMÉ

La production de vivoplants de bananier et plantain indemnes de pathogènes est une étape importante pour rendre possible l'utilisation en plein champ du matériel végétal comme semences saines, exemptes de toute infection bactérienne ou virale. L'objectif de cette étude était d'évaluer l'effet de différents substrats sur la reprise des fragments de bananier indemne de virus et de bactérie pathogènes et la croissance des plantules en vue d'une multiplication rapide des rejets. Un essai a été installé suivant un dispositif de split plot à deux facteurs avec trois répétitions. Le premier facteur était le substrat à trois niveaux (Sciure de bois blanc, Tourbe de coco+sable et Sciure de bois blanc+effluent de manioc) et le second facteur était le cultivar à quatre niveaux (Aloga, Planta, Sotoumon et Gunkoékoé), donnant au total 12 traitements. Les cultivars utilisés sont indemnes de virus et de bactéries pathogènes. Les résultats ont révélé que l'apparition des bourgeons dépend du substrat et du cultivar. La tourbe de coco+sable et Sciure de bois blanc+effluent de manioc ont significativement influencé ($P<0,001$) les paramètres de croissance et le rendement en plantules des bananiers. De plus, le substrat témoin qui est la sciure de bois blanc a induit le plus faible nombre de plantules, 60% de moins que la tourbe de coco+sable. L'ensemble des résultats obtenus dans ce travail indique que la tourbe de coco+sable est efficace pour la production rapide du matériel végétal de plantation du bananier par la méthode de Plants Issus de Fragments (PIF).

Mots Clés : Effluent de manioc, tourbe de coco, sciure de bois blanc

INTRODUCTION

Expended production of banana and plantain in sub-Saharan Africa is greatly hindered by scarcity of quality seed (Kasyoka *et al.*, 2010). Propagation of banana is, thus done in the traditional way by suckering, a practice with disadvantages such as the relatively long time to obtain, the low yield of suckers, the heterogeneity of the suckers and the large volume of plant material (Bangata *et al.*, 2018). This multiplication technique is a potential means of and spreader of pests, such as nematodes, weevils, bacterial, fungal and viral diseases (Delgrange, 2003).

The quest for an adequate and rapid multiplication of suckers from healthy banana plants has led to consideration of a diversity of methods, such as production of vitroplants and vivoplants (Gandonou *et al.*, 2012; Koné, 2013; Koné *et al.*, 2016). *In vitro* methods make it possible to obtain sufficient disease-free improved plant materials. However, most of the techniques are too technical to producers because of high cost (Youmbi and Ngaha, 2004).

In vivo methods, on the other hand, have shown effectiveness and are yet more accessible to producers in Africa (Njeri *et al.*, 2012). The only exception among these techniques is plants from stem fragments (PIF), through which it is possible to quickly produce plant materials of the vivoplant type. This method is more farmer friendly and, thus accessible to producers who, after three to four months, as young ready-to-plant banana plantlets could be obtained (Bonte *et al.,* 1995; Kwa, 1998). Although the PIF technique brings relief to producers, it continues to be studied owing to the diversity of cultivars (Bangata *et al.*, 2019; Kouakou *et al.,* 2019). The nature of these substrates is crucial because they constitute the support on which the small fragments grow, which therefore need special care to produce vigorous seedlings.

In Benin, some banana cultivars respond very poorly to the PIF technique because of the type of inappropriateness of the substrate used (Atekpami, 2017). This implies that the success of this PIF technique for a given cultivar depends on the type of substrate. There is, therefore need for testing successful usable substrates in order to obtain optimal production of healthy suckers for cultivar multiplication. The objective of this study was to evaluate the effectiveness of different substrates on the recovery of fragments and the growth and number of seedlings per explant obtained by the PIF technique of different banana cultivars.

MATERIALS AND METHODS

Plant materials. The banana cultivars used in this study are presented in Table 1. The choice of these cultivars was based on their popularity among small scale farmers, traders, consumers and especially for the medicinal value associated with some of them (Fassinou and Olounladé, 2017). Additionally, the choice of cultivars in the field took into account the absence of virus symptoms on suckers weighing 200 - 500 g.

After rigorous selection of these suckers, they were soaked in a disinfectant solution with ash plus water, for 20 minutes in order to eliminate fungi and residual bacteria, so as to obtain healthy fragments (Kwa, 2003).

The set up. The experiment was carried out in well-secured hotbeds built from local materials (bamboo). Each sprouter had a length of 2.20 m, width of 1.80 m and a height of 0.6 m. Each sprouter included 12 equal compartments separated into three batches, with a distance of 20 cm between two consecutive batches. Each set included four lockers or compartments. All the germinators were raised by 0.45 m from the ground using, pilings to avoid contamination from direct contact of substrates and the ground beneath the germinators.

The experiment was set up in a two-factor split plot arrangement. The main factor was the substrate at three levels, namely: white sawdust, coconut peat + sand and white sawdust + cassava effluent. The minor factor was the four-tiered banana cultivar (Aloga, Planta, Sotoumon and Gunkoékoé). The main factor (substrates) was allocated in main plots to avoid the border effects resulting from watering two different substrates laid-out side by side. The levels of these factors were combined to form factorial treatment combinations (Table 2), replicated three times.

A total of 12 treatments (Table 3), repeated 3 times, were installed, i.e. 36 elementary plots (3 substrates x 4 levels of cultivars x 3 repetitions). Each plot unit contained three explants, each one spaced 10 cm apart in a plot. The distance between two plots was 20 cm; while that between two blocks was 30 cm. The control substrate (white sawdust), is the substrate usually used by the producers of young seedlings of banana for all cultivars in Benin.

The depth of the germinators was 40 cm in order to facilitate the rooting of the sown fragments. The first watering of the sprouter

TABLE 1. Characteristics of banana cultivars used in the evaluation of growth substrates

Cultivar	Characteristics
Aloga	Plantain banana with long fingers, very yellow interior; vegetation cycle of 10 to 12 months; cultivar able to give two bunches; fruit is hard even when the pulp darkens. The fruit can be made into fries, crisps, pounded dough, flour.
Planta	Dessert banana that retains its green colour when ripe with medium-sized large fingers has vegetation cycle of 8 to 10 months.
Sotoumon	Dessert banana of pure yellow colour when ripe has short fingers; is sweeter than all other dessert bananas has a vegetation cycle of 8 to 10 months. Its fruit can be transformed into donuts commonly called ''Talé-Talé''.
Gunkoékoé	Dessert banana with larger and slightly longer fingers than Sotoumon has a light yellow colour when ripe has a vegetation cycle of 8 to 10 months. Its fruit is also used in the manufacture of therapeutic remedies.

TABLE 2. Treatments and structure as applied to the study

Factors	Levels
Substrates	White sawdust (S1)
	Coconut peat + fine sand (S2)
	White sawdust + Cassava effluent (S3)
	Cultivar 1: Aloga (C1)
Cultivars	Cultivar 2: Planta (C2)
	Cultivar 3: Sotoumon (C3)
	Cultivar 4: Gunkoekoe(C4)

TABLE 3. The processing operations considered and their acronyms

No.	Treatments	Acronyms
1	T1= White sawdust * Cultivar 1	S1C1
2	T2=White sawdust * Cultivar 3	S1C3
3	T3= White sawdust * Cultivar 4	S1C4
4	T4= White sawdust * Cultivar 2	S1C2
5	T5= Coco peat + fine sand * Cultivar 4	S2C4
6	T6= Coco peat + fine sand * Cultivar 1	S2C1
7	T7= Coco peat + fine sand * Cultivar 2	S2C2
8	T8= Coco peat + fine sand * Cultivar 3	S2C3
9	T9= White sawdust + Cassava effluent * Cultivar 3	S3C3
10	T10= White sawdust + Cassava effluent * Cultivar 4	S3C4
11	T11= White sawdust + Cassava effluent * Cultivar 1	S3C1
12	T12= White sawdust + Cassava effluent * Cultivar 2	S3C2

was done 24 hours after sowing; while subsequent waterings was done based on humidity. Thus, 2 to 3 weekly watering intervals were done during the 60 days of the trial. The constant presence of water droplets under the transparent plastic paper was an assurance that the germinator maintained normal growth conditions.

Data collected and parameters calculated. The data collected during the experiment included plant height, leaves per seeding, leaf length and width, diameter at neck and number of roots per young seedling Plant height was measured using a tape measure from the collar to the last leaf. Both leaf length and width were used to calculate leaf area using Kumar *et al.* (2002) procedure:

$$STF = L*La*0{,}8*N*C$$

Where:

SFT = total leaf area of the plant,
N = total number of leaves,
L = length of third youngest leaf,
La = width of third youngest leaf, 0.80 = proportionality factor proposed by Murray (1960),
C = coefficient of the new factor (C= 0.662 if the number of leaves in the exponential growth phase varies from 1 to 2,

C = 0.4; if the number of leaves in the exponential growth phase varies from 3 to 30).

Statistical analyses. Data were subjected to a two-way analysis of variance using SAS software (version 9.4). Significant effects, treatment means were separated using Turkey's test at a level of significance of P<0.05.

RESULTS

Rate of recovery of fragments. The time taken to observe the appearance of buds on the fragments of each cultivar depended on the substrates (Table 4). After sowing, Aloga and Planta cultivars showed buds on white sawdust + cassava slurry and coco peat + fine sand substrates, respectively, in eight days. Planta and Gunkoékoé cultivars, on the other hand, showed buds appearing with a delay of four days, compared to those in white sawdust substrate (Control substrate), where the first buds for the Aloga and Gunkoékoé cultivars appeared after the double number of days of white sawdust substrates + cassava effluent and coconut peat + fine sand. On the other hand, the Planta and Sotoumon cultivars induced the appearance of buds about three weeks after sowing.

Growth parameters

Number of leaves. The interaction between substrates and cultivars were not significantly different (P = 0.33) for number of leaves (Fig. 1). Substrates composed of coco peat + sand, and white sawdust + cassava slurry significantly (P < 0.05) increased the number of leaves over the white sawdust substrate (Table 5). The number of leaves obtained was not significantly (P > 0.05) different among cultivars (Table 6). On the other hand, the application of the substrates on the fragments significantly (P< 0.001) influenced the number of leaves of the seedlings whatever the cultivar.

Number of roots. There was interaction between substrates and cultivars on the number of roots (Fig. 1). There were more roots of banana and plantain seedlings of: Aloga cultivar on the substrate White sawdust + cassava effluent, Gunkoékoé cultivar on coconut peat + sand, Sotounmon cultivar on coconut peat + sand, Aloga cultivar on coconut peat + sand, Planta cultivar on coco peat + sand, Planta cultivar on white sawdust + cassava effluent (Fig. 1). On the other hand, the lowest values (7.47) were obtained in Aloga cultivar on white sawdust, Gunkoékoé cultivar on white sawdust, Soutoumon cultivar

TABLE 4. Comparison between the effectiveness of the substrates tested, the appearance of the first buds on each cultivar

Substrates	Cultivars	Latency time* (d)
White sawdust	Aloga (C1)	15
	Planta (C2)	20
	Sotoumon (C3)	20
	Gunkoekoe (C4)	15
Coconut peat + sand	Aloga	8
	Planta	12
	Sotoumon	8
	Gunkoekoe	12
White sawdust + cassava effluent	Aloga	8

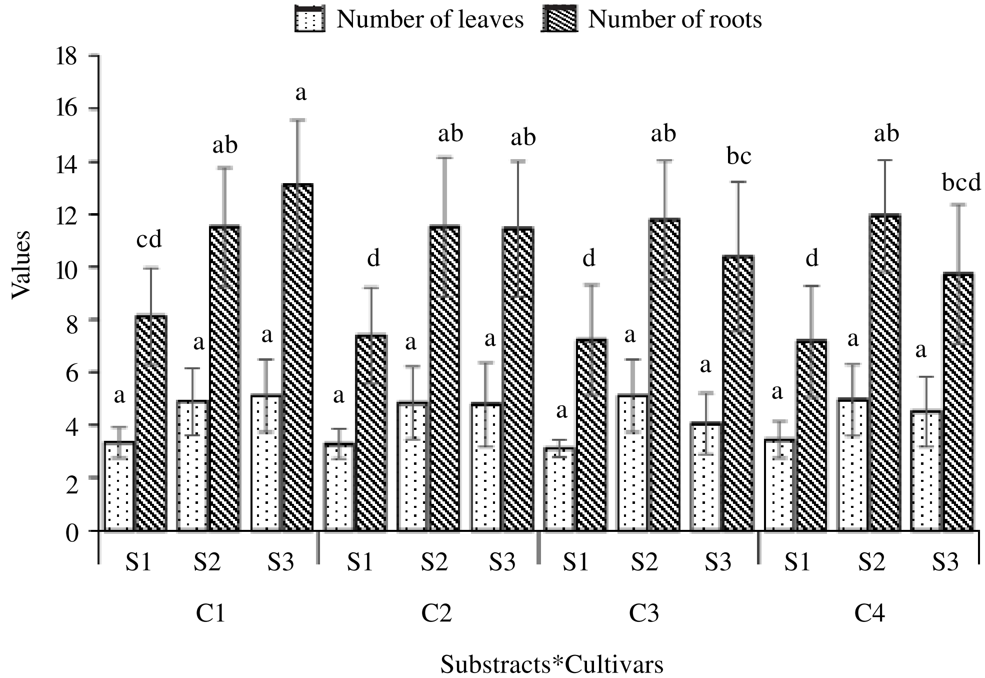

Figure 1. Performance of substrate type and cultivars on number of leaves and roots of banana and plantain seedlings. Histograms bearing the same alphabetical letter are not significantly different at the 5% level.

TABLE 5. Leaf and root number under the influence of substrate type

Substrates	Number of leaves			Number of roots		
	Mean	Standard error	Group	Mean	Standard error	Group
White sawdust	3.29	0.07	B	7.47	0.23	B
Coconut peat + sand	4.94	0.16	A	11.68	0.27	A
White sawdust + Cassava effluent	4.61	0.16	A	11.16	0.34	A

For the same factor and for the same variable, the values accompanied by the same letter are not significantly different at the 5% level (Turkey's test)

on white sawdust and Planta cultivar on white sawdust.

The different substrates strongly ($P < 0.001$) influenced the number of roots of the plantlets (Table 5). There was no significant difference ($P > 0.05$) between the number of roots on the three test cultivars (Table 6). The coco peat + fine sand and white sawdust + cassava resulted in more ($P < 0.05$) roots than in the white sawdust (Table 5). The greatest number of roots was obtained in the Aloga, Planta and Sotoumon cultivars (10.91, 10.11 and 9.80, respectively) (Fig. 1).

Leaf area. The interaction between substrates and cultivars was significant ($P < 0.05$) on leaf area of seedlings (Table 7). Seedlings grown in the white sawdust substrate had the

TABLE 6. Number of leaf and root under the influence of banana and plantain cultivars

Cultivars	Number of sheets			Number of roots		
	Mean	Standard error	Group	Mean	Standard error	Group
Aloga	4.44	0.19	a	10.91	0.41	a
Planted	4.3	0.2	a	10.11	0.41	Ab
Sotoumon	4.09	0.18	a	9.8	0.42	Ab
Gunkoekoe	4.3	0.18	a	9.61	1.41	B

For the same factor and for the same variable, the values accompanied by the same letter are not significantly different at the 5% level (Turkey's test)

TABLE 7. Leaf area under the influence of banana and plantain seedling cultivar

Cultivars	Mean	Standard error	Group
Aloga	965.22	94.61	A
Planta	890.38	95.39	A
Sotoumon	613.16	88.86	B
Gunkoekoe	775.44	85.18	AB

For the same factor and for the same variable, the values accompanied by the same letter are not significantly different at the 5% level (Turkey's test)

lowest leaf area. Thus, the combination of the Aloga cultivar with White sawdust + cassava effluent; Planta cultivar on coco peat + sand and on the white sawdust + cassava effluent; Gunkoékoé cultivar on coco peat + sand; Sotoumon cultivar on coco peat + sand had the highest leaf areas (Fig. 2). On the other hand, small leaf surfaces were obtained on white sawdust + Planta.

Plant height and diameter at the collar of the seedlings. The interaction between substrates and cultivars had a significant effect (P>0.05) on the height and the diameter at the collar of the banana and plantain seedlings (Table 8). Thus, the interactive effect of the Aloga cultivar on white sawdust + cassava effluent provided the highest value of height and diameter at the collar of the seedlings (Fig. 4). Coco peat + fine sand and white sawdust + cassava effluent substrates also showed similar effects on these parameters on the three test cultivars. However, the shortest plants were obtained with the interaction of Planta cultivar and white sawdust; and that of diameter with the Sotoumon cultivar on white sawdust (Figs. 3 and 4).

DISCUSSION

Rate of recovery of fragments. The appearance of buds as a response to exposure of banana and plantain plantlets cultivars depended on the time duration of exposre to different substrate types (Table 4). The results demonstrate that coco peat + sand and white

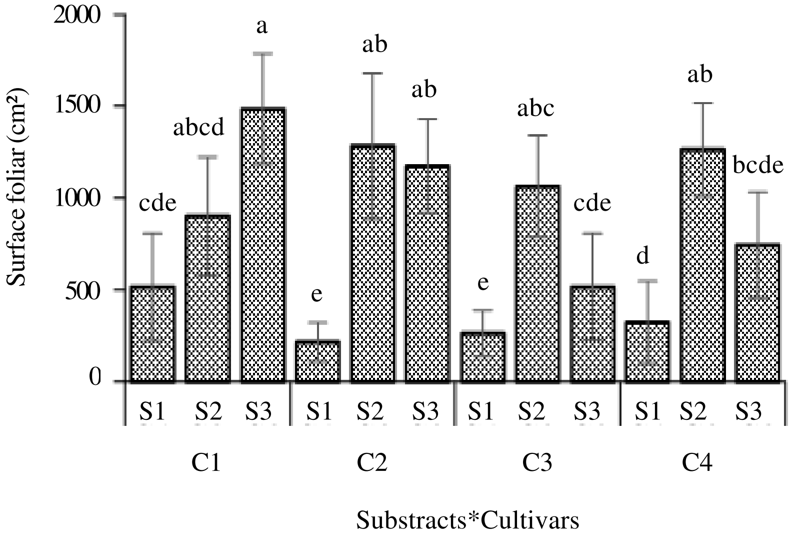

Figure 2. Interactive effect of substrate type and cultivars on leaf area of banana and plantain plants. Histograms bearing the same alphabetical letter are not significantly different at the 5% level.

TABLE 8. Plant height and diameter at the root of banana and plantain seedlings under the influence of cultivar

Cultivars	Height (cm)			Collar diameter (cm)		
	Mean	Standard error	Group	Mean	Standard error	Group
Aloga	41.86	1.55	a	3.01	0.1	A
Planta	37.22	1.47	b	2.58	0.11	B
Sotoumon	34.14	1.37	b	2.33	0.11	B
Gunkoekoe	35.79	1.43	b	2.45	0.11	B

For the same factor and for the same variable, the values accompanied by the same letter are not significantly different at the 5% level (Turkey's test)

sawdust + cassava effluent caused the buds of banana and plantain shoots to appear significantly ($P<0.05$) earlier (8 to 12 days) than in white sawdust (15-20 days). This may be attributed to the quality of the substrate. Compared to the results of Kwa (2003), which revealed that the latency time of the different cultivars tested varies from 21 to 28 days on white sawdust, the results of the present study revealed a shorter latency time for the white sawdust substrate and more efficient for coconut peat + sand and white sawdust + cassava effluent.

The longest latency times were observed with the Planta and Gunkoékoé cultivars (Table 4). On the other hand, the Aloga and Sotoumon cultivars were the first to react by producing buds. This reactivity was different depending on the cultivars, because budding is a genetic characteristic linked to the cultivars (Coulibaly, 2014).

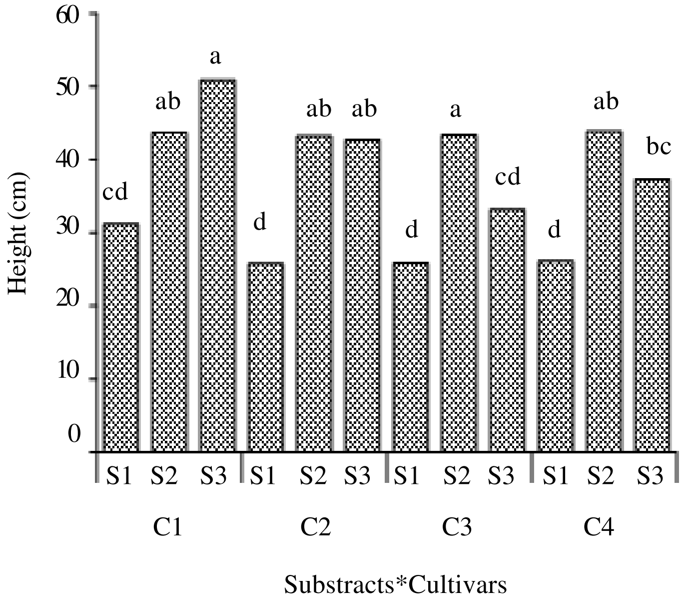

Figure 3. Interactive effect of substrate type and cultivars on the height of banana and plantain seedlings. Histograms bearing the same alphabetical letter are not significantly different at the 5% level.

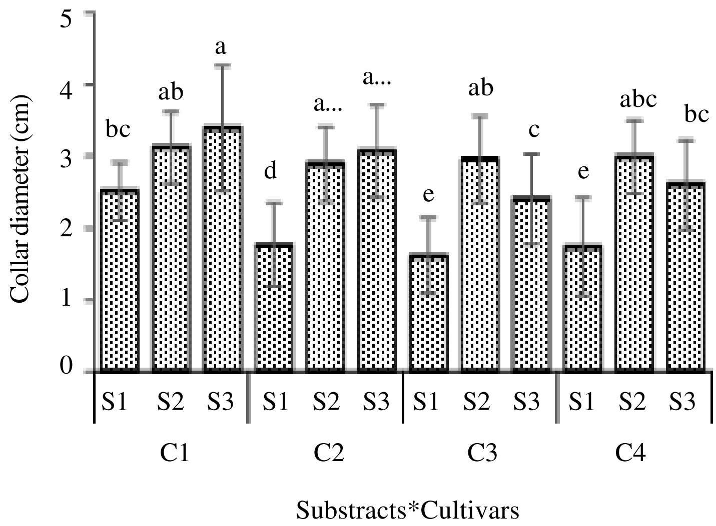

Figure 4. Interactive effect of substrate type and cultivars on banana and plantain seedling collar diameter. Histograms bearing the same alphabetical letter are not significantly different at the 5% level.

These results are similar to those of Koné (2013), who conducted work with the seedbed of the fragments to awaken the buds and stimulate the recovery of seeds at the time of germination. This recovery required the mobilisation of reserves for the development and growth of the buds before their emergence above the surface of the substrates.

The performance of banana plantlets on a new medium may be defined from the physiological point of view, by its capacity to survive and grow by obtaining a higher yields than that of the plants cultivated in a classic medium (Slama *et al.*, 2005).

Number of leaves. The reaction of banana and plantain suckers to the substrates used was manifested during the first stages of recovery by the appearance of the number of leaves, which varied signifcantly between substrate types (Fig. 1).

The majority of plants growing in coco peat + sand and white sawdust + cassava effluent substrates produced six to seven leaves per plant (Fig. 1). Plants in white sawdust, on the other hand, produced three to five leaves per plant. The result obtained for white sawdust corroborates with those of Kwa (2003), where by the suckers produced three and five leaves per plant. The results obtained in the present study differed from the work of Kwa (2003) with regard to coconut peat + sand and white sawdust + cassava effluent, possibly due to the porosity and good water holding capacity of these two substrates which supplied seedlings properly with water and nutrients, especially nitrogen, which is capable of stimulating leaf growth (Nyembo *et al.*, 2012).

Number of roots. In terms of roots produced, the most numerous ($P < 5\%$)) were found in plants grown in the substrates coconut peat + sand and white sawdust + cassava effluent (Fig. 1). These results may again be attributed to the porosity and water holding capacity of these substrates which promote root development. For example, the coco peat + sand is an excellent medium capable of promoting good aeration and normal water retention; all useful conditions for rapid growth of the roots and the plant in general. The results relating to coconut peat + sand agree with those of Bongoua-Devisme *et al.* (2018), who showed that incorporation of coconut fiber (product derived from coconuts such as coco peat) into the soil (sand) improves the structure of the soil and makes it more permeable and well aerated. This allows better root development and, therefore, better growth.

Leaf area. Leaf area was also influenced by the interaction between substrate types and banana cultivar used (Table 7). Substrates coconut peat + sand and white sawdust + cassava effluent induced the highest leaf areas, contrasting with white sawdust which had the lowest leaf areas. This could be attributed to the high rate of organic matter in the substrates (coconut peat + sand and white sawdust + cassava effluent), which facilitated better porosity, better water retention capacity which favored the leaf growth of the seedlings having grown on these substrates. Similar results were obtained by Shahina *et al.* (2012) and Hamidi *et al.* (2017), who also highlighted the significant effect of organic-based substrates on leaf number and leaf area.

Plant height and diameter at the collar of the seedlings. Plant height and circumference of the seedlings were greater ($P<0.05$) in coconut peat + sand and white sawdust + cassava effluent compared to the white sawdust (control). This result suggests that the coco peat + sand and white sawdust + cassava effluent are suitable to the growth of seedlings (Table 9). This growth can be associated with several reasons, namely: these substrates have a pH 6.45 to 6.60; at this pH, most mineral nutrients contained in the soil are made available to the plant (FAO, 2005).

TABLE 9. Plant height and diameter at the collar of banana and plantain seedlings under the influence of the type of substrate

Substrates	Height (cm)			Collar diameter (cm)		
	Mean	Standard error	Group	Mean	Standard error	Group
White sawdust	27.21	0.78	b	1.91	0.08	B
Coconut peat + sand	43.52	0.9	a	2.99	0.06	A
White sawdust + Cassava effluent	41.03	1.23	a	2.87	0.09	A

For the same factor and for the same variable, the values accompanied by the same letter are not significantly different at the 5% level. (Turkey's test)

CONCLUSION

From this study, coconut peat plus sand and that of white sawdust plus cassava effluent stand out as the most suitable substrates for rapidly multiplying disease free banana seedlings from micro-fragments. Overall however, coconut peat plus sand is the best because all the cultivars respond favourably to it. Nevertheless, Aloga and Gunkoékoé cultivars had better performance on white sawdust + cassava effluent. Further studies could deepen the understanding of the physico-chemical compositions of the different substrates in order to further explain the role of these different components on the growth parameters as well as on the number of young seedlings of banana.

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