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Original Article

Branch Diameters, Substrates, and Indole-3-Butyric Acid Effect on Cashew (*Anacardium occidentale* L.) Tree Propagation by Air Layering

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ABSTRACT

Ivorian cashew (*Anacardium occidentale* L.) orchards are essentially comprised of unselected trees of diverse origins and characterized by a very low yield per hectare. In these orchards, potentially high-yielding trees have been identified. Vegetative propagation remains the most appropriate technique for the regeneration of these trees. Air layering is the regeneration technique used for a number of wood species that are difficult to root through clonal propagation. The objective of this study was to set up a regeneration protocol using the layering technique. Specifically, we evaluated the effect of the substrates, diameter class, and indole butyric acid concentration on the rooting percentage and survival of hardened plants. For this purpose, two diameter classes 5–8 mm and 8–12 mm, four substrates (sawdust, rice chip, coco peat, and coffee integument), two concentrations of indole butyric acid (2.5 and 5 mg/L) and one control (no indole butyric acid solution). were tested. Rooting percentages of 88.09, 79.36, and 61.90 were obtained with sawdust, rice chip, and coco peat respectively. No roots were observed on the coffee integument. Diameter class 8–12 mm promoted the most significant percentage of survival (89.41%) and rooting (85.18%). The concentration of 5 mg/L favored the induction of the greatest number of roots (30.79). The specific percentage of possible survival in the field of the weaned layer was above 80%. Branches with 8–12 mm diameter treated with indole butyric acid 5 mg/L in sawdust induced was the optimum treatment combination for cashew nut layering propagation in Côte d'Ivoire.

Keywords: Air layering, *Anacardium occidentale* L., branch diameter, IBA, rooting substrate

Introduction

Cashew is native to Brazil and cultivated for its fruit (Trevian et al., 2005). Its nuts are used in food industry, cosmetics, medicine, and the automotive industry (Aliyu & Awopetu, 2007). Cashew cultivation has contributed to the socioeconomic development of several countries like Côte d'Ivoire, Cameroon, and Ghana (Bezerra et al., 2007).

The increase in the market value of cashew nuts on the Ivorian market from 150 FCFA in 2006 to 500 FCFA in 2018 has stimulated the planting of the crop, particularly in the Côte d'Ivoire. In 2018 Ivorian cashew nut production was 738 000 t, making it the highest-producing and exporting country in the world (FIRCA, 2018). Such production has been made possible through the extension of cultivated areas, which increased from 500 000 ha in 2006 to around 1 350 000 ha in 2018 (FIRCA, 2018). This extension of cultivated areas has caused problems of the availability of cultivable land and exacerbated local food insecurity.

Despite leading world production, the nut yield of Ivorian orchards remains low (350 to 500 kg/ha) compared to the minimum yield of Indian orchards which is between 2 to 6 t/ha (FIRCA, 2018). This reduced productivity is principally due to the creation of plantations by seedlings (Fe & Aklobessi, 2018). Surveys in Ivorian orchards have identified 209 potentially high-yielding genotypes (Kouakou et al., 2020). These potentially high-yielding trees can form a nucleus resource for cashew tree genetic improvement. Vegetative propagation is the most appropriate technique for capturing and replicating these selected trees (Kanmegne et al., 2015). The most used techniques are grafting, cutting, and layering. Grafting does not allow the faithful reproduction of the mother plant because the rootstock is different from the mother plant (Djaha et al., 2019; Kambou et al., 2019). Cutting offers the possibility to faithfully reproduce the mother plant, unfortunately the cuttings are recalcitrant to rooting (Kouakou et al., 2021).

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Layering is frequently presented as a solution to the vegetative propagation of wood species that are recalcitrant in rooting. This method has been employed successfully for the propagation of *Ficus carica* L. (Reddy et al., 2014) and *Anacardium occidentale* L. (Aliyu, 2007; Shetty & Melanta, 1990). The rooting response to layering is influenced by the diameter of the branches (Mialoundama et al., 2002; Paluku et al., 2018), the concentration of indole butyric acid (IBA), and the substrate of culture (Patel et al., 2012). Little information is available on the layering of the Ivorian cashew tree. The objective of this study is to develop a protocol for the vegetative propagation of *Anacardium occidentale* mature selections through layering. Specifically, it aims to determine the effect of rooting substrate, branch diameter class, and IBA concentration on percent adventitious root production and clone survival post weaning for cashew layering.

Experimental Site

This experiment was carried out from March to September 2021 in Abidjan, Côte d'Ivoire, at University Nangui Abrogoua (05°23´N, 04°00´W). The experimental station is established in the forest zone with four distinct seasons, two dry (December to March and July to August), and two wet (April to June and September to November). The annual average rainfall varies between 1800 and 2000 mm. Mean monthly temperature varies between 27°C and 30°C, whereas mean relative humidity ranges between 70% and 84%. The soil of the site is of ferralitic type and strongly desaturated. The soil pH is more acidic at the surface than at depth, and the organic matter content varies from 2 to 3% (Kimou et al., 2017).

Plant Material

Branches with diameters 5–8 mm and 8–12 mm constituted the plant material for this study. The two classes of diameter were selected in order to obtain plants of 30 to 40 cm in length. Because plants of small size are easy to transport, branches were selected from 5-year-old trees located on cashew germplasm of the University Nangui Abrogoua.

Substrate Types

Four substrates were used in this study (coffee integument, rice chip, sawdust, and coco peat) (Figure 1). Coco peat is a well-draining substrate with a low risk of root asphyxia, good water retention

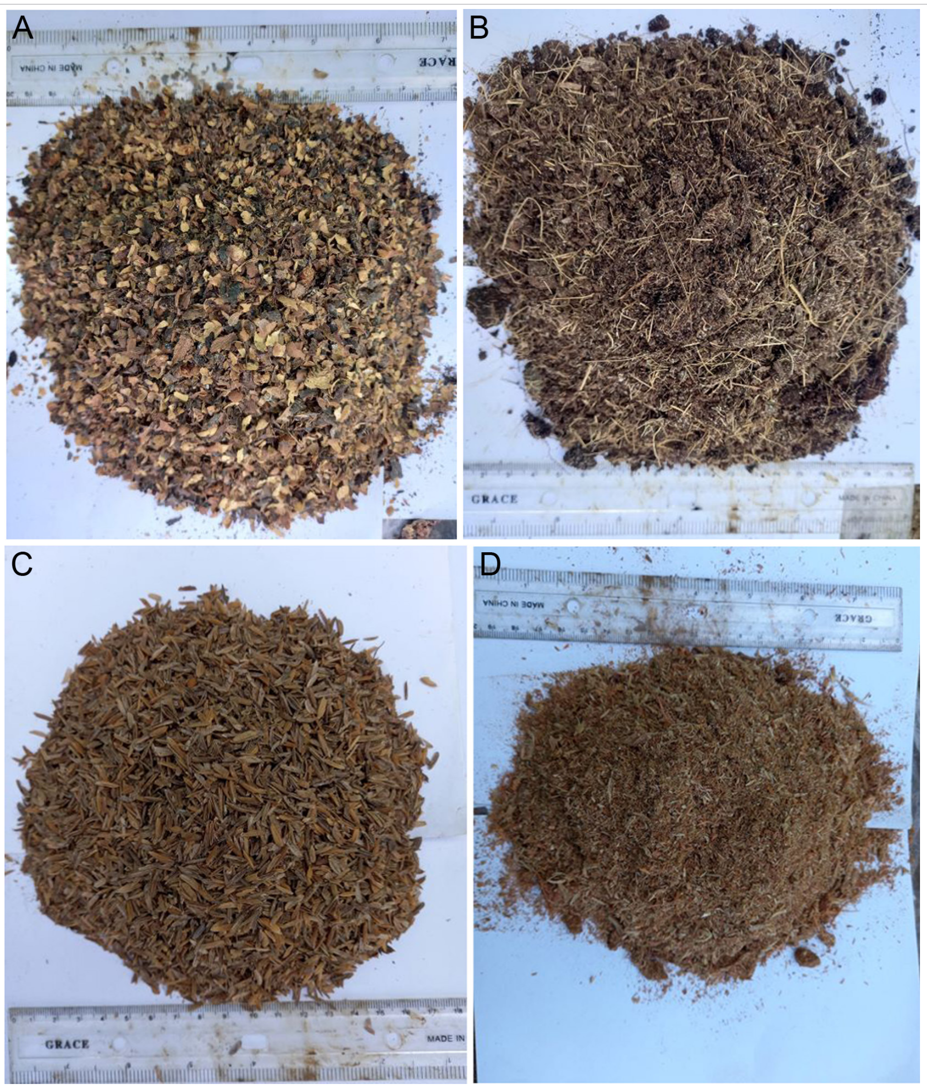

Figure 1.
Substrates Used for Layering: (A) Coffee Integument; (B) Coco Peat; (C) Rice Chip; (D) Sawdust.

Figure 2.
Layering Steps: (A) Ringed Branch; (B) Branch Layered.

capacity, and good structural stability. Sawdust absorbs water easily and maintains humidity for good plant growth. the rice chip has a low water retention capacity and is very aerated. Coffee integument has a less draining substrate.

Methods

The study was conducted in a randomized block design with three repetitions in a 4 × 2 × 3 factorial scheme. The factors are represented by two classes of branch diameter (5–8 mm and 8–12 mm), four substate types (coffee integument, coco peat, sawdust, and rice chip), and two concentrations of indol-3-butyric acid(IBA) (2.5 mg/L and 5 mg/L) and one control (no IBA solution). Each of the three blocks consisted of 24 experimental units, individually composed of a plant. Each treatment was performed separately in the plants.

The layering technique employed is that described by Moupela et al. (2020). It consists of letting roots form while the stem is still attached to the mother plant. It is only after the roots have formed that the layer is detached and planted in the ground.

Layering Technique

Vigorous and healthy branches with a diameter comprised between one of the two classes indicated were randomly selected around the four quadrants of the plants. Selected branches were leafed over 10 cm (Figure 2). A ring of bark measuring 3 to 5 cm in length was removed from under a node 25 or 30 cm from the apical part of the branch with a knife sterilized with 70% ethanol. Each girdle was wrapped entirely with 400 mL of one of four substrates, which was moistened with 200 mL distilled water containing one of two IBA treatments (2.4 and 5 mg/L) and control (water only) and wrapped in transparent polyethylene bags 13 cm × 35 cm. Air layers were identified with labels containing information regarding the treatments. One month after applying the layers, 30 mL of water was added weekly to each substrate using a medical syringe to prevent the substrate from drying out.

Weaning and Monitoring in Nursery

Layers revealing adventitious roots visible under the surface of the plastic wrap 60 days after laying were removed by cutting the branches about 3 to 4 cm below the layer using well-sharpened and disinfected secateurs (Figure 3). The layers were transported to the nursery where the leaf area was reduced by 50%. The plastic wrap was removed from the layers while retaining the substrate root ball. The layers were then transferred to black polyethylene nursery bags of size 30 cm × 20 cm previously filled with topsoil. Layers were transferred and maintained under a greenhouse with 80% shade during the nursery period. Layers were watered the same day and then watered every 2 days.

Planting and Layer Monitoring in Field

After a month of weaning, the layers having produced new leaves were transplanted to the fields (Figure 4). The planting was carried out in holes of dimensions 40 cm x 40 cm on the experimental plot of the University Nangui Abrogoua. Plants spacing was 2 m × 2 m. After planting, plants were watered, and regular weeding was done around the plants during the monitoring.

Data Collection

The parameters recorded during data collection were average root length, average number of roots, average root diameter, rooting percentage, and survival percentage.

Figure 3.
Weaning Stages: (A): Layer Ready to Be Weaned; (B) Layer Stripped of Plastic; (C) Layer Transferred to a Nursery Bag.

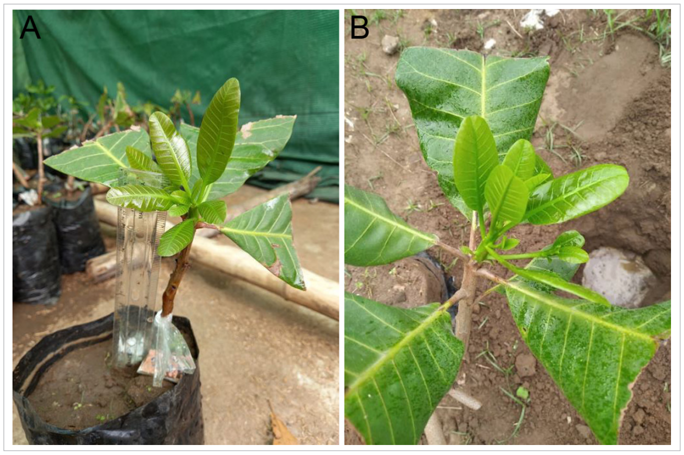

Figure 4.
Transplanting Process of Layer: (A) Successful Layer in the Nursery; (B) Transplanted Layer.

Number of Roots
This was settled by counting the number of roots that had been formed after root initiation.

Average Root Length
Three individual roots were selected and their lengths were determined and average root length was fixed by adding the three root lengths and dividing the value by three. The ruler utilized was a 30 cm ruler.

Average Root Diameter
Six individual roots were selected, and their diameters were determined. The average root diameter was determined by adding the six root diameters and dividing the value by six. Calipers were used to measure the diameters of the roots.

Percentage Survival of Layers
This was determined by visually assessing the branches that had survived over the total number of branches air layers among the various treatments used and multiplied by 100.

The Percentage of Layers Rooting
This was determined by visually assessing branches that had rooted over the total number of branches air layered among the various treatments used and multiplied by 100.

The percentage of layers survival in nursery: This was determined by visually evaluating the layers that had produced new leaves over the total number of layers in the nursery among the various treatments used and multiplied by 100.

Statistical Analysis
The experimental data obtained were subjected to analysis of variance (ANOVA) and Student's *t*-test using STATISTICA 7.1 software. The Student's *t*-test was used to compare the influence of the two diameter classes on the parameters studied. Analysis of variance was used to compare the effect of substrates and IBA concentrations on the parameters studied. When a significant difference is observed between treatments for a parameter, the ANOVA is completed by the Newman Keuls test at the 5% level for the classification of the different means.

Results

Effect of Substrates on Root Growth, Percentage Survival, and Percentage Rooting of Layers
According to Table 1, the lowest percentage of layer survival was observed with coffee integument. No rooting percentage was obtained with the coffee integument. Thus, the highest rooting percentage was obtained with sawdust (88.09%). The highest mean root number was obtained with sawdust (29 ± 1.3). As for the mean root length and the mean root diameter, coco peat and rice chip showed the highest mean.

Effect of Indole-3-Butyric Acid on Root Growth and Percentage Rooting of Layers
The different treatments (concentration of indole butyric acid (IBA) and control) applied resulted in root induction (Table 2). The percentage of rooting increases with IBA concentration. The highest percentage of rooting (87.3 %) was obtained with the 5 mg/L IBA. The lowest rooting percentage was obtained with the control. The highest mean root length (8.11 cm) and root number (31 ± 1.87) were produced with the 5 mg/L concentration. The highest mean root diameter was obtained at a concentration of 5 mg/L of IBA.

Effect of Branch Diameter on Root Growth, Percentage Survival, and Percentage Rooting of Layers
Branch diameter influenced the percentage of survival and rooting of layer (Table 3). Branch diameters of 8–12 mm favored a survival and rooting percentage of 89.41% and 85.18% respectively. Branch diameters of 8–12 mm yielded a higher mean root number (28 ± 1.23) and root diameter (3.67 mm) than branch diameters of 5–8 mm. The mean root length was significantly identical regardless of the diameter class used.

Combined Effect of Branch Diameters, Indole-3-Butyric Acid, and Substrates on Rooting Ability
The combined effect of treatments influenced all parameters evaluated (Table 4). Indeed, the combination of sawdust and 5 mg/L of IBA

Table 1.
Influence of Substrate on Root Growth, Percentage Survival, and Percentage Rooting of Layers

Substrate	Mean Root Length (cm)	Mean Number of Roots	Mean Root Diameter (mm)	Percentage Survival of Layers (%)	Percentage Rooting of Layers (%)
Sawdust	6.22 ± 0.87^b	28.88 ± 1.3^a	3.06 ± 0.03^b	93.65 ± 1.06^a	88.09 ± 2.25^a
Coco peat	7.48 ± 1.75^a	20.70 ± 1.44^c	3.49 ± 0.02^a	65.87 ± 2.86^c	61.90 ± 2.78^c
Rice chip	8.20 ± 1.75^a	24.83 ± 1.4^b	3.55 ± 0.07^a	80.95 ± 2.91^b	79.36 ± 2.57^b
Coffee integument	NR	NR	NR	28.56 ± 2.18^d	NR
p	<.001	<.001	<.05	<.001	<.001

Means followed by the same letter in a column are not significantly different at $p = .05$.
NR = not rooted.

Table 2.
Influence of Indole-3-Butyric Acid on Root Growth and Percentage Rooting of Layers

IBA Concentrations (mg/L)	Mean Root Length (cm)	Mean Number of Roots	Mean Root Diameter (mm)	Percentage of Rooting (%)
Control	6.84 ± 1.37[b]	17.83 ± 1.32[c]	3.02 ± 0.34[a]	68.25 ± 3.77[c]
2.5	6.95 ± 0.98[b]	25.66 ± 1.05[b]	3.31 ± 0.45[a]	73.80 ± 2.34[b]
5	8.11 ± 1.8[a]	30.79 ± 1.87[a]	3.43 ± 0.49[a]	87.30 ± 2.40[a]
p	<.05	<.001	.094	<.05

Means followed by the same letter in a column are not significantly different at $p = .05$.
IBA = indole butyric acid.

Table 3.
Influence of Branch Diameters on Root Growth, Percentage Survival of Layers, and Percentage Rooting of Layers

Branch Diameter Class (mm)	Mean Root Length (cm)	Mean Number of Roots	Mean Root Diameter (mm)	Percentage Survival of Layers (%)	Percentage of Rooting (%)
5–8	7.23 ± 0.54	19.84 ± 1.55[b]	2.95 ± 0.40[b]	54.89 ± 22.23[b]	50.72 ± 2.81[b]
8–12	7.37 ± 0.51	27.55 ± 1.23[a]	3.67 ± 0.48[a]	89.41 ± 16.13[a]	85.18 ± 1.19[a]
t	0.320	4.086	2.568	3,502	3.204
p	.749	<.01	<.05	<.001	<.01

Means followed by the same letter in a column are not significantly different at $p = .05$.

Table 4.
Combined Effect of Substrates, AIB Concentration, and Branch Diameter Class on Roots Growth, Percentage Survival of Layers, and Percentage Rooting of Layers

Substrates	IBA Concentration (mg/L)	Branch Diameter Class (mm)	Mean Root Length (cm)	Mean Number of Roots	Mean Root Diameter (mm)	Percentage Survival of Layers (%)	Percentage Rooting of Layers (%)
Coco peat	0	5–8	5.93 ± 0.33[d]	14 ± 1.52[d]	2.71 ± 0.2[c]	57.14 ± 14.28	57.14 ± 14.28
	0	8–12	6.70 ± 0.15[cd]	15 ± 1.15[cd]	3.35 ± 0.11[bc]	52.38 ± 4.76	52.38 ± 4.76
	2.5	5–8	7.03 ± 0.96[c]	19 ± 2.51[c]	3.63 ± 0.30[b]	47.62 ± 12.59	47.62 ± 12.59
	2.5	8–12	6.16 ± 0.33[d]	23.33 ± 2.33[b]	3.02 ± 0.04[bc]	76.19 ± 4.76	52.38 ± 4.76
	5	5–8	10.10 ± 0.63[a]	25.75 ± 3.05[c]	3.14 ± 0.09[bc]	66.66 ± 4.76	66.66 ± 4.76
	5	8–12	8.96 ± 0.39[ab]	27.12 ± 3.78[b]	3.02 ± 0.27[bc]	95.23 ± 4.76	95.23 ± 4.76
Sawdust	0	5–8	6.3 ± 0.43[d]	20.5 ± 3.50[c]	3.31 ± 0.04[bc]	80.95 ± 4.76	71.42 ± 8.24
	0	8–12	5.56 ± 0.23[de]	21.33 ± 1.20[c]	3.22 ± 0.05[bc]	95.23 ± 4.76	95.23 ± 4.76
	2.5	5–8	6.23 ± 0.40[d]	29.33 ± 1.45[b]	3.17 ± 0.1[bc]	90.47 ± 9.52	85.71 ± 8.24
	2.5	8–12	7.36 ± 0.08[b]	28.33 ± 1.76[b]	3.72 ± 0.42[ab]	100.00 ± 0.0	85.71 ± 0.00
	5	5–8	6.7 ± 0.41[cd]	34 ± 0.88[ab]	3.52 ± 0.23[b]	95.23 ± 4.76	90.47 ± 4.76
	5	8–12	5.16 ± 0.08[e]	40.33 ± 3.71[a]	3.98 ± 0.04[a]	100.00 ± 0.0[a]	100.00 ± 0.0[a]
Rice chip	0	5–8	7.2 ± 0.46[bc]	17.33 ± 1.45[c]	2.63 ± 0.12[c]	38.09 ± 4.76	38.09 ± 4.76
	0	8–12	9.36 ± 0.32[ab]	19.33 ± 6.56[c]	2.92 ± 0.14[bc]	95.23 ± 4.76	95.23 ± 4.76
	2.5	5–8	7.66 ± 0.29[b]	26.66 ± 0.88[b]	2.97 ± 0.14[bc]	80.95 ± 4.76	80.95 ± 4.76
	2.5	8–12	7.23 ± 0.68[b]	27.33 ± 2.18[b]	3.35 ± 0.12[bc]	90.47 ± 4.7	90.47 ± 4.7
	5	5–8	9.16 ± 0.33[ab]	28 ± 4.72[b]	3.08 ± 0.09[b]	80.95 ± 4.76	71.4 ± 8.24
	5	8–12	8.6 ± 0.05[ab]	30.33 ± 2.72[b]	3.83 ± 0.35[ab]	100.00 ± 0.0[a]	100.00 ± 0.0[a]
p			.010	.002	.018	.000689	.013646

IBA = indole butyric acid.

produced the greatest number (40.33) and diameter (3.98 mm) for branch diameters from 8 to 12 mm. Furthermore, the longest were obtained with layers cultivated with coco peat treated with the 5 mg/L IBA regardless of branch diameter (8.96 and 10.10 cm mean root for 5–8 and 8–12 cm branch diameter class respectively). The percentage of survival and rooting were obtained with branches of diameter 8–12 mm whatever the substrate used.

Combined Effect of Branch Diameters and Indole Butyric Acid on the Percent of Survival of Layers in the Nursery

Regardless of branch diameter class, the percentage of weaning success was highest and statistically identical when treated with IBA, unlike the control which records the lowest percentage (54.04 et 58.24%) (Figure 5). Layers with a diameter of 8–12 mm and treated with 5 mg/L of AIB resulted in a survival percentage of 83.24.

Combined Effect of Branch Diameters and Indole Butyric Acid on the Percent of Survival of Layers in the Field

The combined effect of branch diameters and IBA concentrations did not influence the layer's survival in the field compared to the control (Figure 6). The percentage of survival, in the field, exceeds 86%, regardless of the treatment.

Discussion and Conclusions

Sawdust provided the best percentage of survival and rooting. These results could be explained by the physical and chemical properties of

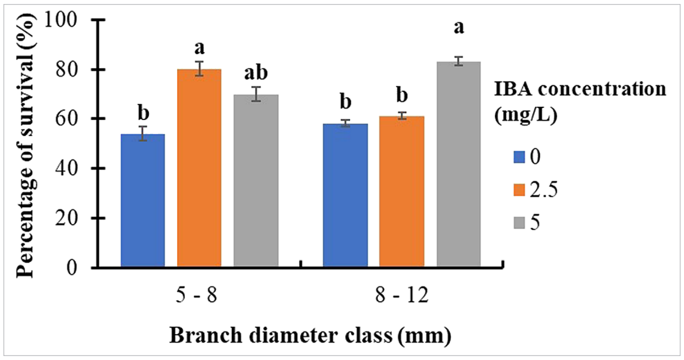

Figure 5.
Influence of the Interaction of Layer Diameter and IBA Concentration on Percentage of Nursery Survival.

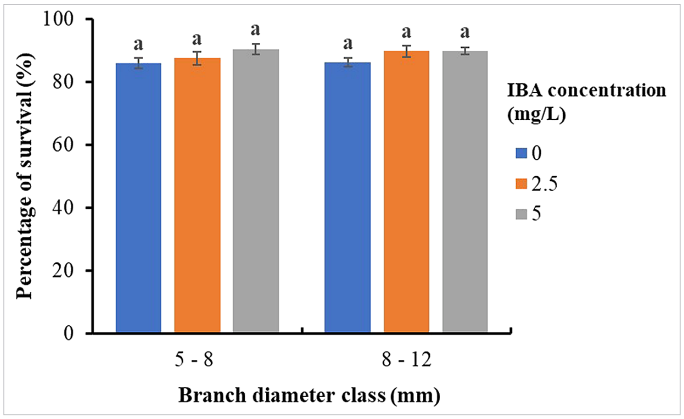

Figure 6.
Interaction Effect of Branch Diameters and IBA Concentrations on Percentage of Layers Survival in Field.

the substrates. The sawdust has a high water retention capacity which would maintain a fairly high humidity around the branches favoring the rooting of the brackets. These results are more pleasing than those of Anliyu (2007) who obtained a rooting percentage of 47% with sawdust. No rooting was observed with the coffee integument. The significant substrate effects observed in percent survival (28.56–93.65%) and percent rooting (0–88.09%) reveal that layering success is influenced by substrate quality. The non-induction of roots by the coffee integument could be explained by the mediocre quality of this substrate. This pasty aspect of the substrate may have adversely affected the cells of the ringed part of the branch, which inhibited roots induction. Our results are contrary to those of Nguema et al. (2013) who show that the air layering ability of *Pseudospondias microcarpa* (A. Rich.) Engl. is independent of the media used. For these authors, the capacity for air layering in this species would be dependent on its intrinsic capacity to respond positively to air layering.

The rice chip and sawdust recorded the greatest number of roots. The highest root diameter and length were obtained with rice chip and coco peat. These results could be explained by the texture of the substrate. Indeed, coco peat and rice chips have a coarser texture and are therefore airier than sawdust. Our results are like those of Mialoundama et al. (2002) who showed that the texture of the sawdust promotes the initiation and growth of roots in the sleeve during the layering of *Santiria trimera* (Oliv.) Aubrév.

Branches of 8 to 12 mm in diameter present a high survival (89.41%) and rooting percentage compared to small diameter (5–8 mm). Large-diameter branches induce thicker and more numerous roots than small-diameter branches. Small-diameter branches can be less tolerant of girdling because it could lead to the destruction of young branch tissues. Large-diameter branches can be more suitable for rooting because they have more nutrient reserves (Kengue and Tchio, 1994). These results have already been observed in *Cola acuminata* (P. Beauv.) Schott and Endl. Paluku et al. (2018) who recommend the use of large-diameter (4 and 4.9 cm) branches for layering.

The concentrations of IBA used in this study promote rooting percentage of layers and the growth of induced roots. The highest rooting percentage, number, length, and diameter were obtained with the IBA 5 mg/L treated layers. The wound operated in the bark of the branch certainly facilitated the penetration of exogenous auxin into the tissues of the branch. This favored the induction of the roots in this zone. According to Cameron (1970) the application of IBA reinforces the photosynthates accumulated at the root initiation site following girdling with *Pinus radiata* (D.Don) H.J. Lam. Our results are contrary to those of Rodrigo et al (2005). These authors obtained a low percentage of survival of the branches treated with AIB compared to the control. The concentration of AIB used by these authors would be too high thus leading to the mortality of the branches.

Sawdust mixed with AIB 5 mg/L on 8–12 mm diameter branches resulted in survival and rooting percentage of 100%. This treatment would be optimal for the success of cashew layering.

Weaning is one of the crucial steps for the production of plants by layering. This operation provokes a change of regime in the life of the layer, in order to assure its survival (Bita, 2016).

The percentage of successful layers in the nursery varies from 54% to 83% depending on the diameter of the layers and the concentration of IBA. The lowest percentage of success was achieved with the small-diameter layers and not treated with IBA. Indeed, these layers characterized by a limited number of roots cannot ensure the survival of layers during weaning. The dieback of layers in the nursery can be attributed to insufficient root development (Jaenicke and Beniest, 2003). The number and quality of the adventitious roots produced by the layer are decisive for its survival in the nursery and the field (Bellefontaine ,2010). However, the most sizeable percentage of success registered in the field can be explained by sufficient weaning in the nursery.

This study revealed that cashew layering is influenced by substrate, branch diameter, and IBA concentrations. Indeed, among the four culture substrates (sawdust, rice chip, coco peat, and coffee integument) no roots were observed on the coffee integument. The most significant percentages of rooting and survival were observed in sawdust and rice chip. These two substrates are suitable for cashew tree layering. Large-diameter branches (8–12 mm) produced a significant percentage of survival and rooting, and induced a considerable number of thick and long roots. Indole butyric acid promoted root induction and the most elevated concentration was 5 mg/L. in addition, branches with 8–12 mm diameter treated with IBA 5 mg/L in sawdust induced was the optimum treatment combination for cashew nut layering propagation in Côte d'Ivoire. It would be important to evaluate the effect of the various factors studied on the development of layering in fields.

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