



## Vegetative propagation of *Anonidium mannii* (Oliver) Engler & Diels (Annonaceae) by leafy stem cuttings in Kisangani, DR Congo

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### Abstract

This study tested the influence of substrate type, size surface and application of IBA auxin on rooting of stem cutting leaf of *Anonidium mannii*, a wild fruit species with low seed germination rate. Two trials were conducted. The first tested three substrate types that are sand, wood sawdust and rice husks. We also tested combinations of these substrates (2:2), resulting in six treatments in a randomized complete block design. The second experiment compared different cutting leaf surfaces (12.5, 25 and 37.5cm<sup>2</sup>) and auxins (IBA applied and not applied) in a split plot design. Using sand as substrate resulted in significantly higher rooting rates (62.1 ± 5.9%), while use of rice husks, even combined with other substrates, did not achieve any cutting rooting. Significant and non-significant differences were observed, respectively, with factors leaf area and auxin application. Highest rooting rates (26.70 ± 6.6%) were obtained with a leaf surface of 37.5cm<sup>2</sup> in combination with IBA application. Vegetative cutting propagation is possible for *A. mannii*, albeit with low rooting rates. Therefore, more targeted testing is required; addressing other parameters such as cutting type, season of cutting and increase of the leaf surface of cuttings.

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## Introduction

Agriculture was started when man became sedentary and focused on stocking food. In the wetlands of tropical Africa, forest ecosystems are still reservoirs of an extremely rich animal and plant biodiversity (Aubé, 1996). The latter is used by is al populations for nutritional, medicinal, socio-economic and cultural purposes (Mbolo, 2002), often without any sustainable management objectives or methods, however (Bonannée, 2003).

Besides timber forest products (TFPs), these forests are also rich in non-timber products (NTFPs; Peter, 2000) consisting of fruits, nuts, seeds, leaf, barks and stems (Wong *et al.*, 2001) which are used by humans (Dupriez *et al.*, 1987). The latter are mostly harvested from wild stands but not from actively cultivated plants (Degrande *et al.*, 2002). However, the domestication of these species could be worthwhile and undertaken by anyone (Dupriez *et al.*, 1987).

*Anonidium mannii* (Oliv.) Engl. and Diels, a fruit tree in the Congo basin rainforest belonging to the family *Annonaceae* is a local species of interest. Its stem can reach high of 30 m and a diameter of 80cm. Its leaves are evergreen, alternate, and simple, up to 45cm long and 18cm wide. Fruits are compound, yellow, surface-crosslinked, weighing 4-10kg. The fruits a high number of brown seeds which are embedded an orange-yellow pulp which represents 60% of total weight. This pulp is high in proteins (Vivien *et al.*, 1996; Lejoly *et al.*, 2010). The fruit of *A. mannii* is an important food in the Tshopo Province of DR Congo, and the bark is used in traditional medicine (Evarest, 2008; Termote, 2012).

Despite all the advantages of *A. mannii*, esp. for the forest population of DR Congo in general and of the Kisangani area in particular, there is no record of this fruit tree being cultivated. The products of this plant are derived from trees wild stands in the surrounding forests which are often subject to deforestation by human activities (Carpe 2001; Bwama *et al.*, 2008), resulting in a decline of this are therefore compelling. Since local people are able to identity suitable trees for cultivation (especially in terms of fruit taste),

vegetative propagation would allow the selection of these genotype and thus maintain preferential characters, excluding low germination rate of seeds (Vivien *et al.*, 1996).

For vegetative propagation by cuttings, several factors can help to promote rooting of the cuttings: type, length and leaf area of cuttings, and type of rooting substrate. These factors have been studied for several species in the Central African Region. Regarding species *Allanblackia floribunda* Oliv. (Clusiaceae), in terms of substrate, highest rooting rates were achieved with sand ( $18.7 \pm 1.3\%$ ; Antangana *et al.*, 2006). For *Dacryodes edulis* (G. Don) HJ Lam. (Burseraceae), wood sawdust and combination sand and sawdust ( $77.7 \pm 5.6$  and  $78.8 \pm 7.8\%$ , respectively) did not yield significant differences in rooting rates compared to the use of sand who was  $58.8 \pm 10.6\%$  (Mialoundama *et al.*, 2002). Regarding hormone use, Indole Butyric Acid (IBA) auxin improve rooting rate of cuttings compared the effect of other auxins (Naphthalene Acetic Acid, NAA or Indole Acetic Acid, IAA); for both *Pausinystalia johimbe* (K. Schum) Pierre ex Beille, (Rubiaceae; Tchoundjeu *et al.*, 2004) and *Baillonella toxisperma* Pierre (Sapotaceae). A combination of  $75\text{cm}^2$  leaf area x IBA auxin x sand substrate resulted in highest rooting rates for *B. toxisperma* (Ngo Mpeck et Atangana, 2007).

Based on these results, our study aimed to evaluate the influence of substrate type, cutting leaf area and IBA auxin on rooting of cuttings of *A. mannii*.

The objective of this study is to develop a vegetative propagation approach to mass-produce seedlings of *A. mannii* by stem cuttings, in order to make available the plant materials to regenerate this species in the fields.

We hereby tested the following hypotheses: (1) rooting rate of *A. mannii* cuttings depends on substrate type used, esp. in terms of texture, structure; (2) larger leaf area, promote rooting due to higher photosynthetic activity; and (3) the application of IBA auxin stimulates rooting in cuttings of *A. mannii*.

## Materials and methods

### Location

The study was conducted in Kisangani (0°31'N, 25°11'E and 396-427 m) in non-mist propagators in hangar at the Faculty Institute of Agricultural Sciences of Yangambi (IFA-Yangambi).

### Materials

We used uninodales stem cuttings of *A. Mannii* of 4-5cm length. Cuttings were obtained from five adult trees at Mobi (0°22'N, 25°23'E, 428m), a village located 32 km from Kisangani, on Lubutu road in Tshopo Province, DR Congo.

### Methodology

#### Collection, transport and conditioning of cuttings

Cuttings were taken from hardened juvenile stem of branches. Immediately after collection, cuttings were moistened with water, placed in plastic bag and transported on motorbike from harvest site to hangar of experimentation. At hangar, cuttings were trimmed to the required dimensions (length 4-5cm) and leaf area reduced (12.5, 25 and 37.5cm<sup>2</sup>), and then kept in a bucket of water before planting, in order to keep cuttings cool.

#### Propagation in non-mist propagator

Propagation was conducted in non-mist propagator, a wooden frame covered with clear plastic to completely seal the system and make it waterproof. The frame was divided into three compartments of 1m<sup>2</sup> each. The propagator base consisted of a dual layer of polyethylene film on which a thin layer of sand was placed. The sand layer was covered successively with a layer of stones, gravel and rooting substrate, each thick 10cm. Water for the rooting substrate was supplied through capillarity, ensuring a constant humidity of more than 80% and a temperature of 28-30°C, which are favourable conditions for cutting development. A plastic pipe with a diameter of 3cm and a length of 30cm was inserted at the corner of each compartment, on the stone layer, to adjust water levels (Tchoundjeu, 1989).

During the experiment, the leaves of the cuttings were moistened at least once a day using a sprayer, to maintain humidity.

### Experimental setup

#### Experiment 1

##### Influence of substrates type

The experimental design consisted of a randomized complete block (Fig. 1) with substrate type as random variable. Each substrate was replicated three times. Each replicate consisted of 22 cuttings of *A. mannii*, resulting in n = 22 cuttings of reduced leaves x 6 treatments (3 substrates and 3 combinations 2-2) x 3 repetitions = 396 cuttings. Substrates tested were rice husks, wood sawdust (a combination of several species) and river sand, and mixtures of equal volumes of sawdust and sand, sand and rice husks, and rice husks and sawdust. Rice husk and sawdust were chosen as they are abundant in the area and are used in gardening. River sand was used in several studies and produced good results for many species; it is readily available in Kisangani town.



**Fig. 1.** Cuttings of *A. mannii* installed in non-mist propagator.

#### Experiment 2

##### Effect of leaf area and IBA auxin on cutting rooting

The experimental design was a split-plot with three levels of leaf area (12.5, 25, 37.5cm<sup>2</sup>) and two levels of IBA auxin (applied, not applied). The combination of these factors resulted in six treatments (Table 1). Each treatment was replicated three times and 30 cuttings were used per treatment, resulting in a total of n = 30 cuttings x 6 treatments (3 leaf surfaces and 2 auxin levels) x 3 replicated = 540 cuttings. Cuttings from sample trees were distributed equally over treatments.

As rooting medium, we used sand. The hormone used was Rhizopon<sup>®</sup> AA (ACF Chemiefarma NV Netherlands, Approval No. 4726), a powder containing 2% of butyric beta-indole acid (IBA).

IBA was applied by putting the bases of the cuttings in contact with the powder (standard application).

Substrate sterilisation was performed by heating on metal half-cask. The substrate was rotated every time water added to ensure good heat distribution in the material.

**Table 1.** Combination factors experimented.

Leaf area	IBA auxin	experimental Treatments
12.5cm <sup>2</sup> (S1)	Treatment (H1)	H1S1
	No treatment (Ho)	HoS1
25cm <sup>2</sup> (S2)	Treatment (H1)	H1S2
	No treatment (Ho)	HoS2
37.5cm <sup>2</sup> (S3)	Treatment (H1)	H1S3
	No treatment (Ho)	HoS3

*Data collection*

Observations on rooting cuttings began two weeks after the setup of cuttings and were performed every week for 22 weeks. We reported the number of cuttings that had lost their leaves; rooted cuttings; number and length of roots per rooted cutting; and number of parched cuttings. Roots were counted and measured when rooting was first observed, using a graduated lath ( $\pm 0.1\text{cm}$ ). Rooted cuttings were withdrawn (Fig. 2) from the propagation chassis when roots reached a length of 1.5cm. Cuttings were then placed in a substrate consisting of compost for their growth. They were gradually acclimatised for two weeks in a large propagator (Fig. 3) before being exposed to the open air.



**Fig. 2.** *A. mannii* rooted cuttings.



**Fig. 3.** Acclimatization of rooted cuttings in giant propagator (3 x 1 x 1.5m<sup>3</sup>).

*Data analysis*

Collected data were recorded in Excel 2007 and analysed using GenStat 14.1 and SPSS 17.0. Logistic regression models were used to detect differences between treatments in terms of rooting and mortality. Graphs on weekly mortality rates and rooting of cuttings were developed using Excel 2007. To determine the effect of experimental factors on rooting success, data were subjected to analysis of deviance using the General Linear Model of Genstat 14.1.

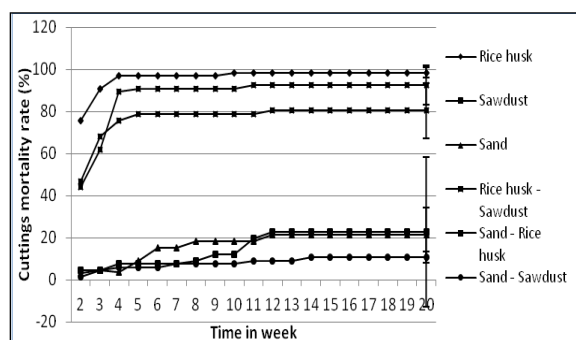
**Results and discussion**

*Experiment 1: Effect of substrate type*

Mortality cuttings of *A. mannii* was relatively high for treatments with rice husks. In the second week, 62% of cuttings in rice husks had already withered. Later on, we observed significant differences ( $P < 0.001$ ) between the different substrates. Sand and sawdust in combination rice husks showed a rather low mortality rate (44-47%) at the beginning of the experiment (second week), while using rice husks without the addition of another substrate resulted in a considerably high mortality rate ( $75.8 \pm 14.6\%$ ) from the beginning on. Mortality rates of cuttings were  $98.5 \pm 2.6$  and  $92.4 \pm 9.5\%$  at the eleventh week for rice husks and sand-rice husks, while rice husks combined with wood sawdust resulted in a mortality rate of  $80.3 \pm 13.1\%$  in week 12.

In sand and sawdust, we observed mortality rates of only  $21.2 \pm 13.1$  and  $22.8 \pm 35.5\%$ , respectively, at the end of the experimental period, whereas the

combination of these two substrates yielded high cutting survival rates ( $10.6 \pm 2.6\%$  mortality). The curves were separated into two batches (Fig. 4).



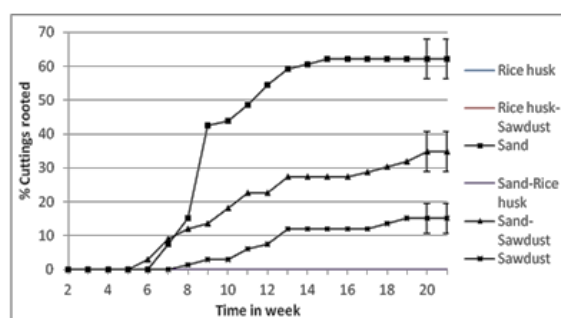
**Fig. 4.** weekly mortality of cuttings of *A. mannii* as function of substrates ( $n=22$ ,  $total=396$ ,  $Deviation=Standard Error$ ).

Similar rates (15-36%) were also recorded by Mialoundama *et al.* (2002) for *Dacryodes edulis* with sandy substrates, sawdust and their combinations. Tchoundjeu *et al.* (2002) also reported a mortality rate of 20% for *Prunus africana* with the use of sawdust as substrate. The lowest rates were reported for *Pausinystalia johimbe*,  $\pm 3\%$  (Ngo Mpeck, 2004; Tchoundjeu *et al.*, 2004) for the same substrate types (sand, sawdust and 1:1 sand-sawdust).

These differences in terms of rooting rates are related to the structure of each substrate, which affects permeability, and water retention and exchange with the atmosphere. Studies by Bourgos Leon in Senegal showed the phytotoxicity of sorghum straw (*Sorghum vulgare*), a cereal grown in Africa, on seedlings or weeds species which grow on this substrate. Phytotoxicity of *S. vulgare* was caused by phenolic acids identified in extracts straw, at concentrations sufficient to inhibit soil properties according to the pedoclimatic conditions of these cultures (Bourgos-Léon, 1975). Rice husks are cereal residues, which was probably the reason for the high mortality rate in our study.

Sand as a substrate resulted in higher rooting rates compared to those obtained for the other substrates ( $62.1 \pm 5.9\%$ ; Fig. 5). By contrast, the treatments with rice husks were not very successful. Treatment with sawdust substrate resulted in  $15.1 \pm 4.4\%$ ; combining

sawdust with sand resulted in increased rooting rates of up to  $34.8 \pm 5.8\%$ . *A. Mannii* cuttings started to root at week 6, earlier than for *Diospyros classiflora* (Hiern) (Ebenenaceae), which started rooting at week 9 under similar conditions (Tsobeng *et al.*, 2011). However, in similar experiments, *Khaya ivorensis*, *Lovoa trichilioides* (Tchoundjeu, 1989) and *Ricinodendron heudelotii* (Schiembo *et al.*, 1997) already started rooting in week 2. In our study, differences between treatments were highly significant ( $P < 0.001$ ) from week 7. Rooting time can be seen as a species-specific function.



**Fig. 5.** weekly cumulative rate of cuttings rooting *A. mannii* as function of six types of substrate ( $n=22$ ,  $total=396$ ,  $Deviation=Standard Error$ ).

The rice husk substrate and its combinations did not result in any rooting, whereas sand was the most successful substrate. High rooting rates in sand have also been reported by Leakey *et al.* (1990), Nyansi (2004), Atangana *et al.* (2006), Ngo Mpeck and Atangana (2007) and Paluku *et al.* (2018) respectively for *Cordia alliodora* (Boraginaceae), *Garcinia kola* (Clusiaceae), *Allanblackia floribunda*, *Baillonella toxisperma* and *Cola acuminata*. Generally, an adequate substrate for rooting cuttings must have an optimum pore volume and allow adequate amounts of oxygen to be respired by the roots (Andersen, 1986). The inferiority of rice husk and sawdust compared to sand would be linked to their level of decomposition, which is often incomplete and continual, resulting in increased heat which impedes growth.

The number of roots per cutting ranged from one to two, and root length ranged from 6 to 10 mm in treatments where rooting was successful (Table 2).

There were no significant differences ( $P > 0.05$ ) between substrates in terms of root number, which is in agreement with previous findings. The number of roots formed per cutting would therefore seem to be species-specific. In terms of root length, sand is significantly different ( $P = 0.028$ ) of combination of sand and sawdust.

**Table 2.** Means number and length of roots ( $n=22$ , total=396, Deviation=Standard Error).

Parameters Substrats	Mean number of roots	Mean length of roots (mm)
Sand	1.4±0.9	6.2±3.4
Rice husk	0	0
Wood sawdust	1.5±0.8	6.2±3.5
Sand-rice husk	0	0
Sand-sawdust	1.2±0.4	9.7±10.3
Rice husk-sawdust	0	0

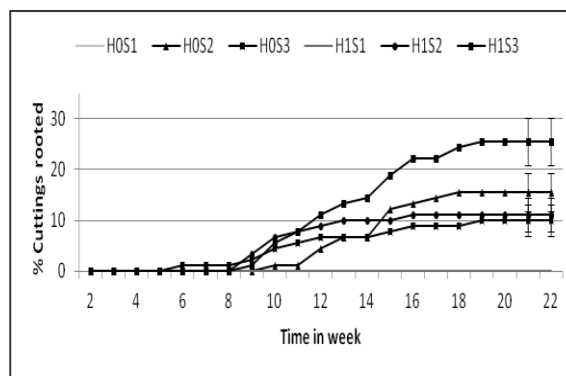
*Experiment 2: Effect of leaf area x auxin IBA*

Cuttings started to root in the sixth week for the treatment without auxin application, and for cuttings with a leaf area of 37.5cm<sup>2</sup>. Highly significant ( $P = 0.003$ ) and very highly significant differences ( $P < 0.001$ ) between treatments were observed at the tenth and the eleventh week, respectively. Rooting rates were positively correlated to a higher leaf area (Table 3). The combination 37.5cm<sup>2</sup> x IBA auxin resulted in highest rooting rates (26.7 ± 6.7%) compared to the other treatments (10-18%). Treatments with 12.5cm<sup>2</sup> leaf area did not result in any rooting (Fig. 6; Table 3).

Results obtained by Tchoundjeu *et al.* (2004) for *P. johimbe* showed that the curve of the rooting rate depends on the foliar surface and is not an exponential, but rather a Gaussian curve (an increase from 0 to 50cm and a decrease between 100 and 200cm<sup>2</sup>). This means there is an optimal, species-specific leaf area on cuttings for obtaining optimum rooting rates. An optimum leaf area for tropical species is one that allows an adequate balance between photosynthetic assimilation and water loss through transpiration (Tchoundjeu *et al.*, 2002 and 2004). Studies on this species, *A. Mannii*, show that leaf area is a determining factor to rooting ability of cuttings in non-mist propagator conditions (Paluku *et al.*, 2019).

**Table 3.** Mean rooting rate (%) of *A. mannii* cuttings as function of leaf area and IBA auxins ( $n=30$ , total=540, Deviation=Standard Error).

Hormone Leaf area	IBA auxin (H1)	No IBA auxin (Ho)	Total Means	
S1=12.5cm <sup>2</sup>	0.00	0.00	0.00	0.00
S2=25cm <sup>2</sup>	10 ± 6.7	17.80 ± 8.4	27.80 ± 7.1	13.90 ± 3.5
S3=37.5cm <sup>2</sup>	26.70 ± 6.7	10 ± 3.3	36.70 ± 10.9	18.35 ± 5.8
Total Means	36.70 ± 13.7	27.80 ± 5.1	12.23 ± 3.3	9.26 ± 4.2



**Fig. 6.** Weekly rooting of cuttings *A. mannii* as function of combination leaf area x IBA auxins ( $H_0 = no\ auxin$ ,  $H_1 = IBA\ auxin$ ,  $S_1 = 12.5cm^2$ ,  $S_2 = 25cm^2$ ,  $S_3 = 37.5cm^2$ ,  $n=30$ , total=540, Deviation=Standard Error).

For the different factors, highly significant differences ( $P < 0.001$ ) were observed between leaf areas and significant differences ( $P = 0.042$ ) between combinations of leaf areas x auxin application, whereas between both auxin treatments, there were no significant differences (Table 4). Numerically (Table 3), application of IBA auxin resulted in higher rooting rates (12.23% against 9.26%). These results corroborate with those obtained by Atangana *et al.* (2006), Ngo Mpeck and Atangana (2007) and Paluku *et al.* (2018), where auxin application (IAA, IBA and NAA) did not improve rooting rate for *A. Floribunda*, *B. Toxisperma* and *Cola acuminata* respectively. In contrast to what occurred with both these three species, increasing doses of IBA auxin (50-300 µg) largely stimulated rooting (at 34 to 87%) in cuttings of *P. africana* (Tchoundjeu *et al.*, 2002). We therefore suggest that responses to auxin application are species-specific.

**Table 4.** Resume of variance analysis of rooting rate of factors and interaction (n=30, total=540).

Variations sources	df	Deviance	Mean deviance	Deviance ratio	Approx. Chi-2 probability
Blocks	2	2.6007	1.3003	1.30	0.272
Auxins	1	2.0119	2.0119	2.01	0.156
Leaf area	2	50.2567	25.1283	25.13	<.001***
Auxins * Leaf area	2	6.3598	3.1799	3.18	0.042*
Residual error	532	298.5681	0.5612		
Total	539	359.7971	0.6675		

\* Significant differences; \*\*\* Very highly significant differences

Correlation analysis showed a correlation between cuttings mortality and leaf loss ( $r^2 = 0.426$ ). There was no correlation between leaf loss and rooting rate ( $r^2 = 0.004$ ), implying that when cuttings lose their leaves, rooting is difficult. These results are in agreement with previous studies (Antangana *et al.*, 2006; Tchoundjeu *et al.*, 2002).

#### Conclusion and perspectives

In order to test the influence of different substrates, leaf area and IBA auxin on rooting cuttings of *A. mannii*, a study was conducted under non-mist propagator conditions in Kisangani (DR Congo).

Substrate characteristics were shown to significantly affect rooting of cuttings. The high rooting rates in sand compared to sawdust or rice husk are possibly related to the substrate's ability to allow water and air to flow freely is those in. Rooting rates evolve in the same direction as the increase in leaf area, involving evolutionary photosynthetic capacities. The IBA auxin slightly influenced rooting success of cuttings *A. mannii*, this may be related to the mode of application and the hormone type used, in relation with the adaptation of the species.

Rooting rates varied significantly between treatments (0-27 and 15-63%) and are possibly related to moment of obtaining the cuttings and experimental period. Plant physiology may also influence cutting capability. To improve rooting success rates, additional studies are needed that should focus on other factors affecting rooting, such as cutting type, season of obtain cutting and increase in leaf surface.

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