Journal of Applied Biosciences 174: 18103 – 18112
ISSN 1997-5902

Propagation of agroforestry tree species by air-layering: a case study of *Canarium schweinfurthii* Engl. in the Western Highlands of Cameroon

***Kanmegne Gabriel, Atchioutchoua S. Raïssa, Tsopmbeng Noumbo Gaston R.**
Department of Plant Biology, University of Dschang, P.O. Box 67 Dschang, Cameroon
*Author for correspondence: gkanmegne@yahoo.fr Tel. (+237) 677 49 00 69

Submitted on 9th May 2022. Published online at www.m.elewa.org/journals/ on 30th June 2022 **https://doi.org/10.35759/JABs.174.6**

ABSTRACT
Objective: As a contribution to the domestication of *Canarium schweinfurthii* (Black olive tree), the present study examined its potential for clonal propagation by air-layering.
Methodology and Results: Three substrates Topsoil, Sawdust and mixture of Sawdust and Topsoil in a 1:1 (v/v) ratio] and five concentrations (0, 1, 2.5, 5 and 10 g/l) of exogenously applied indole butyric acid (IBA) were tested for their effects on the rooting of air-layers. Results showed that the percentage of rooted layers and the mean number of roots per rooted layer were significantly ($p < 0.001$) influenced by substrate and IBA concentration, whereas the layers' mortality rate was influenced only by substrate ($p < 0.001$). The highest percentage of rooted layers (78.57 ± 7.78%) was recorded with the combination of Sawdust substrate and 5 g/l IBA. The same treatment combination resulted in the highest mean number of roots per rooted layer (34.95 ± 0.76). The lowest mortality rate of layers (6 ± 1.94%) was recorded with Sawdust substrate.
Conclusion and application of findings: *C. schweinfurthii* is amenable to vegetative propagation through air-layering technique. For prolific rooting of air-layers, Sawdust substrate and application of 5 g/l IBA are recommended. With the increasing demand for *C. schweinfurthii* fruits, these study findings would contribute to rapid and mass propagation of this multifunctional indigenous tree species which have suffered neglect in research. Selecting trees with desirable traits and propagating them asexually using simple and inexpensive method as described in this work would improve establishment of this plant whose fruits trade raises the living standard of many populations in Western and Central Africa.
Keywords: *Canarium schweinfurthii***,** Domestication, Non-timber forest products, Vegetative propagation, Western Highlands of Cameroon

INTRODUCTION
Domestication and cultivation of indigenous tree species producing high valued non-timber forest products (NTFPs) in agricultural systems is an on-going practice in many developing countries around the tropics and sub-tropics. Domestication of indigenous tree species aims at intensifying and diversifying smallholder farming systems, to increase farmers' income (Atangana *et al*., 2014). Priorities setting exercises and ethno botanic surveys have identified some 50 African agroforestry tree species as priority targets for

domestication, owing to their useful marketable food and non-food products (Franzel *et al*., 2008; Leakey *et al*., 2022). Research studies over the past three decades have developed strategies for the domestication of many of these priority species (Leakey *et al*., 2022). Nevertheless, a number of high valued species among which *Canarium schweinfurthii* have been neglected in research. *Canarium schweinfurthii* Engl. (Fig. 1), a member of the Burseraceae family (APG III, 2009) is one of the many African tree species that are of socio-economic importance. Its common names in English include: Torchwood, Frankincense, Black olive, Bush candle tree and Forest pear (Tcheghebe *et al*., 2016; Anozie and Oboho, 2019). It is a tall, upright tree that grows up to a height of 50 m and a trunk diameter of more than 150 cm in dense evergreen humid forests, semi-deciduous forests, secondary forests, gallery forests and dry forests. The tree is widely distributed throughout the western, the central and the eastern Africa (Orwa *et al*., 2009). In its distribution's area, the plant is extensively used in food, traditional medicine, timber industry, handicrafts, and as firewood. The most used parts of the plant are: fruits, leaves, bark and wood (Traoré *et al*., 2021). As food, the fruit pulp is edible and can be eaten raw or after softening by soaking in warm water. *C. swchweinfurthii* fruits are traded throughout the distribution area of the species and even beyond. Traders buy these fruits in rural areas and sell them in main towns which are the major consumption centers (Njoukam and Peltier, 2002). Although statistics on trade flows are not available, ethno-botanic surveys have revealed that the market of *C. schweinfurthii* fruits significantly increases the income of those involved in its value chain (Tsewoue *et al*., 2019; Traoré *et al*., 2021). The leaves, the bark and the seeds are used in medicinal preparations for the treatment of various pathologies including dysentery, gonorrhoea, cough, stomach problems, skin diseases and high blood pressure (Koudou *et al*., 2005; Okullo *et al*., 2014; Tsewoue *et al*., 2019). The wood and the resin from *C. schweinfurthii* are exploited for industrial and artisanal uses. Indeed, the wood is used for making veneer sheets, plywood, flooring, furniture. In some regions, it is used for the construction of canoes, troughs, planks and mortars. It is also used as fuelwood. The resin is sometimes used as candles, torches or as a smoke bomb to ward off mosquitoes. It is also used for repairing pots, clogging canoes and fixing arrowheads (Tchouamo *et al*., 2000).

Figure 1: *Canarium schweinfurthii* tree

Despite the positive impact of *C. schweinfurthii*'s exploitation in the daily life of populations, its cultivation by farmers is not common, although the species was identified as priority target for domestication (Njoukam and Peltier, 2002; Traoré *et al*., 2021). Previous investigations revealed that the main reason why farmers are discouraged from practicing the cultivation of the species is that there is no certainty that the plant raised from sowed seed or from seedlings transplants will produce fruits. Indeed, the most important factor that determines the value of a *C. schweinfurthii* tree is its fruits production (Nzegbule and Nwachi, 2008; Tsewoue *et al*., 2019). The species is dioecious and only female trees are productive (Njoukam and Peltier, 2002). Thus, seeds produced by the same tree are made up of different genetic combinations and therefore produce genetically different plants: some of which may be female and productive as well as others male and unproductive. During vegetative growth phase which lasts at least eight years, there is no index that makes it possible to distinguish male stands from female stands (Njoukam and Peltier, 2002). An alternative mean to propagate the plant other than by seed could be by vegetative approach. The added value of vegetative propagation techniques is the potential to capture and mass propagate the desired phenotype of selected individual trees. The development of an efficient protocol for vegetative propagation would be an incentive for smallholder farmers and agro-foresters to adopt the cultivation of this species whose exploitation enhance the living standard of people in many areas around tropical Africa. The objective of this study was to investigate the responsiveness of *C. schweinfurthii* to vegetative propagation through air-layering technique. The study determined the influence of different substrates and different concentrations of exogenously applied auxin on the rooting of air-layers.

MATERIALS AND METHODS

Study Site: Air-layering experiment was conducted in Baleng (latitude 5°31'N, longitude 10°24'E, altitude 1370 m), a village located within Bafoussam municipality, in the Western Highlands of Cameroon. The climate of the Western Highlands of Cameroon is of equatorial type, with two seasons: a dry season which extends from mid-November to mid-March and a rainy season which extends from mid-March to mid-November. The average annual rainfall ranges between 1500 and 1800 mm, and the average temperature is between 20 and 30°C.

Marcot Setting and Treatment: In the study site, twenty (20) scattered trees growing in agricultural system were selected and used for the study. The selected trees were those that had already produced fruit at least once and had at least 30 marcottable branches. On each selected tree used as block replicate, 30 branches of 3-4 cm diameter (measured using a calliper) were selected at random in the crown and used for marcot setting. Branches were selected independently of their orthotropic or plagiotropic orientation. On each selected branch, marcot setting was done in June 2019 as follows: 60 cm below the apex of the branch, a ring of bark 10 cm in length was completely removed using a sharp knife (Sthapit *et al*., 2016) (Fig. 2a). The stripped area was imbibed with 10 ml IBA solution at the desired concentration (0, 1, 2.5, 5 or 10 g/l). After hormone application, the stripped area was covered with substrate which was Sawdust, Topsoil or a mixture of Sawdust and Topsoil in a 1:1 (v/v) ratio. The substrate was wrapped in transparent polythene sheet and tied at its two ends with a rubber band (Fig. 2b). At one-week intervals, 50 ml of distilled water was injected into each marcot using a syringe, in order to keep the substrate moist.

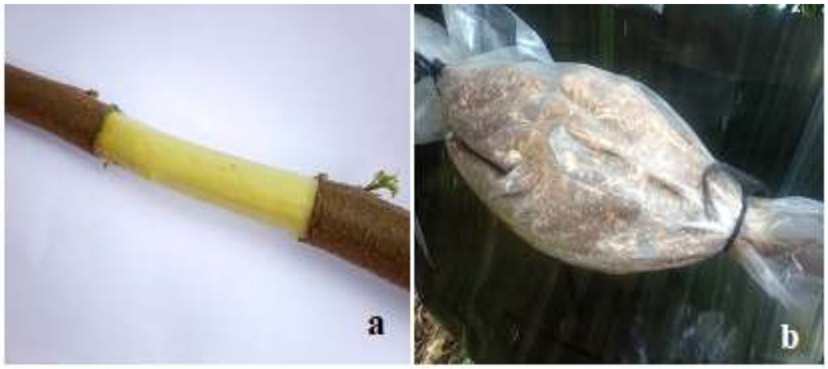

Figure 2: Steps of marcot setting showing a branch with a ring of bark removed (a) and a stripped area of branch covered with substrate, wrapped in transparent polythene sheet and tied at its two ends

Experimental Design: The experiment followed a 3 x 5 factorial experimental layout. There were three substrates (Sawdust, Topsoil and mixture of Sawdust and Topsoil at 1:1 (v/v) ratio), five concentrations of IBA (0, 1, 2.5, 5, and 10 g/l), making a total of 15 treatment combinations. On each parent tree used as block/replicate, two marcots were set for each treatment combination. This made a total of 40 marcots per treatment combination for the 20 selected trees. The total number of marcots which were set for the whole experiment was thus 600 (15 treatment combinations x 2 marcots/tree x 20 trees) and the experimental design was randomized complete blocks.

Data Collection and Analysis: Layers were assessed for rooting at 2-week intervals and the experiment was ended after six months, when there was no further rooting over a period of four consecutive weeks. At the end of the experiment, the following data were recorded for each marcot: survival, rooting success and number of roots. After data collection, the rooted layers were detached from the parent tree, potted in polyethylene bags and reared in the nursery for further experiments. Data were analysed using the General Linear Model of IBM SPSS 21 software package. The variables were mean mortality rate (%) of layers, mean percentage of rooted layers and mean number of roots per rooted layer. Percentage data were transformed into arcsine square root value before analysis. Statistical significance was determined using analyses of variance (ANOVA). Treatment means were separated using Duncan's Multiple Range tests ($p < 0.05$).

RESULTS

Effects of Substrate and IBA Concentration of Percentage of Rooted Layers: Rooting began 16 weeks after marcots setting and continued until the 24th week (six months) after which no further rooting was noted over a period of four consecutive weeks. On each rooted layer, roots emerged from the upper end of the stripped area (Fig. 3).

Figure 3: Rooted *C. schweinfurthii*'s marcot at six months after setting

The results of the analysis of variance (Table 1) showed that substrate and IBA concentration had significant effects on the percentage of rooted layers, whereas interaction of both factors had no significant effect. At six months after marcots setting, the cumulative percentage of rooted layers recorded with Topsoil substrate (32.98 ± 4.87%) was lower than that recorded with either Sawdust (57.45 ± 4.17%) or mixture of Topsoil and Sawdust (49.99 ± 4.27%). These two last substrates were not different from each other for their percentage of rooted layers. The percentage of rooted layers increased in response to IBA application. Application of 5 g/l IBA resulted in 68.42 ± 5.55% rooted layers, which was not significantly different from that recorded with either 2.5 g/l IBA (66.67 ± 5.51%) or 10 g/l IBA (64.86 ± 5.58%), but was higher than that recorded with 0 and 1 g/l IBA. Sawdust substrate x 5 g/l IBA treatment combination resulted in highest percentage of rooted layers (79.31 ± 7.62%) (Table 2).

Table 1: ANOVA's result showing the degree of freedom (*DF*) and the level of significance (*F* and *p* values) of the effects of substrate, concentration of exogenously applied auxin ([IBA]) and interaction of both factors (Substrate X [IBA]) on *C. schweinfurtii* air-layering parameters

Source of variation	**% of rooted marcots**			**Mean roots count**			**Mortality**		
	DF	***F***	***p***	***DF***	***F***	***p***	***DF***	***F***	***p***
Substrate	2	8.86	< 0.001	2	305.77	< 0.001	2	36.93	< 0.001
[IBA]	4	23.99	< 0.001	4	344.95	< 0.001	4	0.24	0.91
Substrate X [IBA]	8	1.01	0.42	7	73.21	< 0.001	8	0.10	0.99

Table 2: Mean percentage of rooted *C. schweinfurthii*'s layers as influenced by substrate and concentration of exogenously applied IBA

[IBA] (g/l)	Substrate			Average
	Topsoil	Sawdust	Topsoil+Sawdust	
0	5.56 ± 2.08	14.81 ± 5.99	11.11 ± 4.73	11.11 ± 3.60^c
1	21.05 ± 9.62	37.93 ± 9.16	28.57 ± 6.88	30.26 ± 5.37^b
2.5	40 ± 8.56	75 ± 8.74	57.14 ± 8.02	66.67 ± 5.51^a
5	42.11 ± 9.58	79.31 ± 7.62	75 ± 9.28	68.42 ± 5.55^a
10	55.56 ± 8.69	78.57 ± 7.78	77.78 ± 8.76	64.86 ± 5.58^a
Average	32.98 ± 4.87$^\beta$	57.45 ± 4.17$^\alpha$	49.99 ± 4.27$^\alpha$	

Note: Within the last column, means ± SE followed by different letters are significantly different at $p< 0.05$; within the last row, means ± SE followed by different symbols are significantly different at $p < 0.05$.

Effects of Substrate and IBA Concentration on the Number of Roots per Rooted Layer: The mean number of roots per rooted marcot was significantly affected by the substrate, the concentration of exogenously applied IBA and combination of both factors (Table 1). When considering the three substrates taken together, the number of roots per rooted layer increased with increasing IBA concentration and reached its highest value (27.40 ± 1.66) at 5 g/l IBA, then slightly decreased to 16.11 ± 0.34 when IBA concentration was increased to 10 g/l (Table 3). In another hand, when considering the IBA concentrations taken all together, Sawdust substrate recorded the highest mean roots count (25.56 ± 1.25), followed by mixture of Sawdust and Topsoil (21.13 ± 1.02) whereas the lowest roots count (6.52 ± 0.42) was recorded with Topsoil. With respect to the interaction of substrate and IBA concentration, the highest mean number of roots per rooted layer (34.95 ± 0.76) was recorded with Sawdust x 5 g/l IBA treatment combination (Table 3).

Table 3: Mean number of roots per rooted *C. schweinfurthii*'s layer as influenced by substrate and concentration of exogenously applied IBA

[IBA] (g/l)	Substrate			Average
	Topsoil	Sawdust	Topsoil+Sawdust	
0	2 ± 0	4 ± 0.41	2.33 ± 0.28	3.12 ± 0.44^d
1	4.75 ± 0.64	7.36 ± 0.61	5.87 ± 0.36	6.39 ± 0.28^c
2.5	5 ± 0.66	34.23 ± 0.76	27.28 ± 0.26	26.64 ± 0.25^a
5	8.46 ± 0.77	34.95 ± 0.76	27.14 ± 0.28	27.40 ± 1.66^a
10	6.37 ± 0.51	20.5 ± 1.02	16.32 ± 0.33	16.11 ± 0.34^b
Average	6.52 ± 0.42$^\gamma$	25.56 ± 1.25$^\alpha$	21.13 ± 1.02$^\beta$	

Note: Within the last column, means ± SE followed by different letters are significantly different at $p< 0.05$; within the last row, means ± SE followed by different symbols are significantly different at $p < 0.05$.

Effects of Substrate and IBA Concentration on the Mortality Rate of Layers: An overall 17.11% of marcots had died at six months after setting, and there were significant ($p < 0.001$) individual effect of substrate on the mortality rate. Neither IBA concentration nor combination of substrate and IBA concentration had significant effect on the layers' mortality rate (Table 1). The mortality rate recorded with Topsoil substrate (37.30 ± 3.96%) was higher than that recorded with either Sawdust (6 ± 1.94%) or mixture of Sawdust and Topsoil (8 ± 2.22%). Sawdust alone and mixture of Sawdust with Topsoil were not different from each other for the mortality rate (Fig. 4).

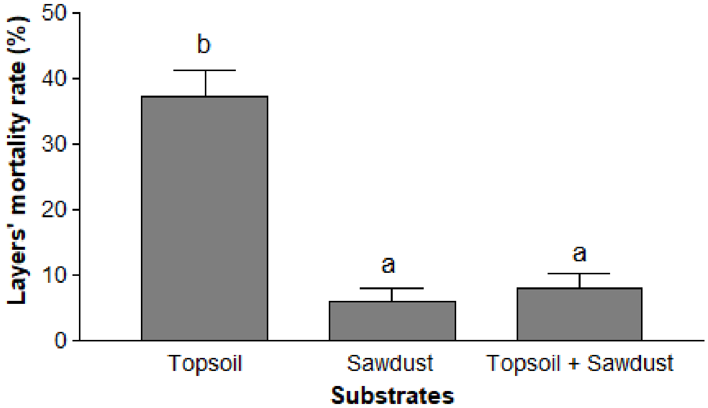

Figure 4: Mortality rates of *C. schweinfurthii* layers with different substrates Bars with same letter are not significantly different at $p < 0.05$

DISCUSSION

The rooting medium (substrate) is one of the key factors that determine the rooting success of air-layers (Lins *et al*., 2015; Bhattacharjee *et al*., 2018; Agarwal *et al*., 2021). From the present study it was noted that Sawdust substrate resulted in the highest success of air-layering (i.e. highest percentage of rooted layer, highest roots count per layer and lowest layers' mortality rate), while the worst performing substrate was Topsoil. These results are consistent with those of Mialoundama *et al*., (2002) and Tsobeng *et al*., (2011) who reported high performance of Sawdust as rooting medium with *Dacryodes edulis* layers and *Diospyros crassflora* cuttings respectively. Moreover, Mahlambi *et al*. (2019) reported poor performance of Topsoil substrate in inducing rooting of *Psidium guajava* air-layers. The variable performances of substrates reported in the present study could be attributed to variable compaction levels of the different substrates tested. Indeed, for successful air-layering of woody plants, media used should be loose and more porous to allow roots emergence and growth (Mishra *et al*., 2017). In this study, Sawdust substrate could favour roots emergence and growth. On the contrary, Topsoil substrate owing to its clay loam texture displayed high level of compaction. Increased level of substrate compaction results in reduced gas diffusion and reduced amount of available oxygen for respiration, all of which inhibit rooting (Huang *et al*., 2012; Agarwal *et al*., 2021). In response to exogenous IBA application, there were significant increases in percentage of rooted layers and mean number of roots per rooted layer. These results were not surprising, since auxins are known to play a key role in the adventitious root formation in woody plants (Leakey, 2014). Indeed, auxins promote cell differentiation, starch hydrolysis, sugars and nutrients mobilization, all of which stimulate roots initiation (Shao *et al*., 2018). Nevertheless, the optimal concentration of exogenously applied auxin needed for prolific rooting varies from a plant material to another, depending on the amount of endogenous auxin already present in the plant tissues (Mukhtar, 2019). The results of the present study clearly indicate that for *C. schweinfurthii* air-layering, 5 g/l was the most suitable concentration of IBA for prolific rooting. This provides evidence for recommending this hormone treatment for the propagation of the species by air-layering.

CONCLUSION AND APPLICATION OF RESULTS

This study reported for the first time that *C. schweinfurthii* is amenable to vegetative propagation through air-layering technique within six months. This is an incentive for researchers and farmers developing nurseries for the domestication of high-value multifunctional agro-forestry tree species indigenous to the tropics and sub-tropics. Selecting trees with desirable traits and propagating them asexually using inexpensive, robust and simple method as described in the present work would improve plantation establishment of this plant whose fruits raise the standard of living of many populations in African countries.

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