

# Impact of Media on Air-layering in the Propagation of *Dennettia tripetala* (Annonaceae), and Its Micro-morphological Characteristics

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## Abstract

A systematic study was carried out on *Dennettia tripetala* to establish an alternative method of propagation and further provide complementary characters for the identification of the species especially in sterile conditions. All methods followed those of previous authors of related studies. Findings from the silvicultural aspect revealed that it is very possible to successfully propagate the species via air-layering, using good quality materials. Top soil performed better than saw dust substrate. The roots appeared and developed much faster than those of the saw dust, before it was harvested and potted. Micro-morphological examination of the foliar epidermises also showed that the species is hypostomatic, while epidermal cells are predominantly polygonal, measuring 37.1 x 22.0 µm and 30.4 x 15.4 µm in size on the abaxial and adaxial surfaces respectively, and with straight to sinose anticlinal walls. Stomata is anomocytic, up to 32.2 x 17.6 µm in size. Wood is vesseless with tyloses present and gums totally absent. Ray multiserate. Pollen grains are predominantly spheroidal with scabrate surface and thin exine. Further studies on other aspects may reveal more useful information about *D. tripetala*.

**Keywords:** conservation; *Dennettia*; propagation; silviculture; taxonomy

## Introduction

Plants have remained integral sources of food and nutrients for both humans and animals. We simply cannot survive without them. These plants contain several chemical compounds essential for the metabolic functioning of our body systems (Achinewhu *et al.*, 1995). *Dennettia tripetala* Bak., family (Annonaceae) is a small tree of the rain-forest and occasionally in the savanna, growing up to 18 m and about 60 cm in girth. Branchless glabrous, leaves oblong or oblong-elliptic, very shortly cuneate at base, obtusely acuminate, about 10-15 cm long and 4-6 cm wide (Hutchinson and Dalziel, 1954; Burkill, 1985; Keay, 1989). It has a limited distribution in Ivory Coast, Southern Nigeria and Western Cameroons (Burkill, 1985); but in recent times, it has been widely domesticated in the Southern, Eastern and Western parts of Nigeria. It is well known as umimi by the Igbo people of Nigeria, Ata Igebere or igberi by the Yorubas, Imako by the Niger Deltas and Urhobo, ako by Bini, Nkarika by the Efik and Ibibo

(Burkill, 1985; Gbile and Soladoye, 2002). The wood is white, soft and not durable, and is susceptible to termites. Bark is fibrous and strongly scented. The young leaves are chewed on account of their pungent spicy taste. The fruits, green at first then turning red, ripen in April and May have a peppery spicy taste and are chewed for this property (Keay, 1989). Okiy (1960) reported that the fruit is regarded as a good source of vitamin.

Studies have shown that *D. tripetala* contains calcium, iron, magnesium, zinc, manganese and copper while the fruit primarily contains minerals, vitamins, oils, iron flavours (Okafor, 1980), crude proteins, fibre, ash, carbohydrate, sulphur (Okafor, 1980, Udoessian and Ifon, 1984; Okwu and Morah, 2004). The leaves have been reported to be useful in treatment of cough, asthma, catarrh, toothache, diarrhoea and rheumatism; and also combined with mango leaves for the treatment of mild (Nwinuka and Nwiloh, 2009). Studies by Ejechi and Akpomedaye (2005) also showed that the fruits of *D. tripetala* contain an essential oil which has been used as an effective preservative for stored grains such as cowpea and maize without negatively affecting their viability.

*D. tripetala* has inconsistent fruiting, poor seed germination and slow seedling growth. However, there is a high demand of its fruit due to its various uses, and hence the species has been rendered threatened in its natural habitat. The seed is the only alternative for its inexpensive propagation. There is dearth information on its macropropagation. This study thus focuses on the macropropagation of this important species, through air-layering in order to achieve an inexpensive domestication and to ensure its continuous availability for human utilization. It also examines the micro-morphological characteristics of the leaf, stem and pollen grain in an attempt to provide additional taxonomic characters for its identification.

## Materials and Methods

### *Marcott preparation and setting*

A fifteen years physiologically matured tree of *Dennettia tripetala* at Eleyele water works in Ibadan metropolis (Latitude 7.4133° N and Longitude 3.8630° E, mean maximum temperature is 26.46 °C, minimum 21.42 °C and the relative humidity is 74.55%) was air-layered using two media (top soil and saw dust). The uniformly sized, selected branches of 12.50 cm (girth) wrapped were left until sufficient roots were visible through the transparent sheet. As soon as roots grow through the ball of moss, such stem was severed using a saw below the girdled area and the plastic sheet was then removed. The marcotts were taken to the nursery and transplanted into polypots filled with top soil for further growth assessment. These seedlings were watered daily.

### *Data collection*

The growth assessment was carried out in Multipurpose Tree Species Multiplication Unit Nursery at Forestry Research Institute of Nigeria (FRIN) located on latitude 7.383333° N and longitude 3.85° E with the main total rainfall of 1548.9 mm falling in approximately 90 days. The mean maximum temperature is 34 °C, minimum 24.2 °C and the relative humidity is 71.9% (Ariwado et al., 2017).

The marcotts were allowed to stabilize for four weeks after which the seedlings were assessed for the number of leaves, height and collar diameter.

### *Statistical analysis*

Data were analysed by analysis of variance (ANOVA). Standard error was used to separate means.

### *Micro-morphological studies*

Fresh specimens of the leaves and stem obtained from the same site of marcott preparation were used for this purpose.

### *Foliar anatomy*

2-5 cm<sup>2</sup> of the leaves were cut and soaked in well covered glass petri-dishes containing concentrated trioxonitrate (v) acid (HNO<sub>3</sub>), for about two hours to macerate the mesophyll. Upon the disintegration of tissues and indication of bubbles, the specimens were carefully transferred unto clean petri-dish and rinsed thoroughly

with distilled water before the epidermises were separated using forceps. Tissue debris was carefully cleared off the epidermises with fine Carmel hair brush, and the isolated epidermal layers were adequately rinsed in water. The epidermises were then transferred into another Petri dish containing 50% ethanol for 1-2 minutes, thereby allowing hardening of cells. Afterwards, tissues were stained with Safranin O for five minutes and then rinsed again in distilled water to remove excess stain. They were mounted in 25% glycerol on clear microscopic glass slides, covered with cover-slips and the edges of the cover slip were ringed with nail varnish to prevent dehydration. Five slides were prepared each for the abaxial and adaxial surfaces of the species, and studied carefully under Olympus light microscope. However, methods followed those of Radford et al. (1974), Adedeji (2004), Chukwuma et al. (2014), and Chukwuma et al. (2017) for leaf epidermal descriptions and Carpenter (2005) for stomata architecture.

### *Stem anatomy*

Fresh stem of *Dennettia tripetala* was used for this purpose. The transverse, radial and tangential longitudinal sections were carefully cut at 10microns using a sledge microtome at the Department of Forest Products Development and Utilization, Forestry Research Institute of Nigeria, Ibadan, Nigeria, following standard procedures. Sections were carefully stained and mounted in DPX mountant, on clear microscopic glass slides and appropriately labelled (Oladipo and Oyaniran, 2013). The mounted specimens were viewed under Olympus light microscope and observations were carefully recorded. Photomicrographic images were also taken with a Scope image 9.0 enabled camera attached to the microscope. All wood anatomical descriptions follow IAWA (1989).

### *Pollen morphology*

Flowers were subjected to pollen analysis using acetolysis method as described by Erdtman (1960). Pollen was described in accordance with Sowunmi (1973, 1995) while shapes and classes were studied and described following Erdtman (1943).

## Results and Discussion

Distributional information sourced from the Forest Herbarium Ibadan (FHI) and University of Ibadan Herbarium (UIH), revealed that the species is confined to the rain-forests of southern Nigeria, extending from Ijebu-Ode in the west to Cross-river in the south. This is an indication that it may not survive in drier parts, hence requiring adequate moisture for optimum growth and productivity (Fig. 1). This distribution pattern had earlier been reported by Keay (1989) and Ayodele and Yang (2012) who noted that the species is typically a forest taxon.

### *Interaction effect of top soil on collar diameter, height and number of leaves of Dennettia tripetala*

Analysis of variance showed the interaction effects of top soil on collar diameter, height and number of leaves of air layered *Dennettia tripetala* cuttings. It was observed that interaction of top soil had significant effect on all the

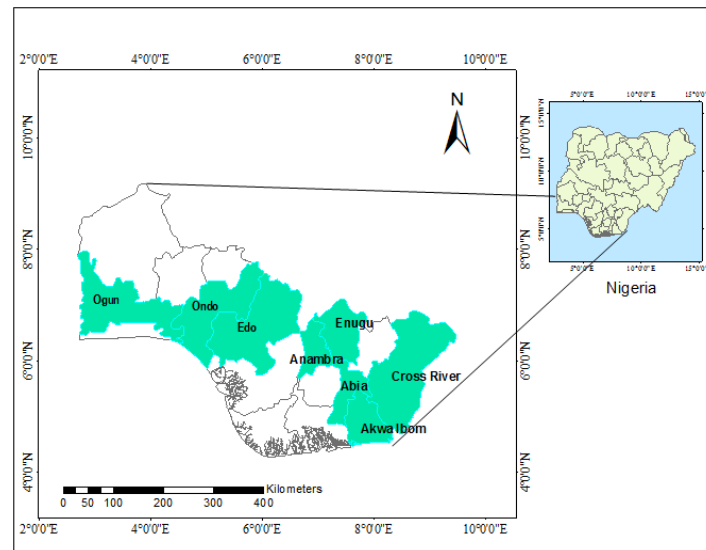


Fig. 1. Map of Southern Nigeria showing distribution of *D. tripetala*

parameters at 0.05 probability level (Table 1). Mean separation showed that collar diameter had the highest value in the month of March for marcotted cuttings from top soil substrate ( $11.2 \pm 0.5$  cm) and the least value in the month of May ( $7.6 \pm 0.7$  cm). Furthermore, height was highest in month May (62.5 cm) for marcotted plant from top soil and least in the month of March (55.4 cm). Number of leaves was highest in the month of May (33) for marcotted plant from top soil and least in February. Number of leaves was highest in the month of May ( $33 \pm 11.6$ ) for marcotted plant from top soil and least in February ( $30 \pm 9.1$ ).

*Interaction effect of saw dust on collar diameter, height and number of leaves of Dennettia tripetala*

The effect of interaction of saw dust on collar diameter, height and number of leaves of air layered *Dennettia tripetala* was also investigated. From the result of analysis of variance, interaction of saw dust had significant effect on all the parameters of air layered *Dennettia tripetala* at 0.05 level of probability (Table 2).

Table 1. Analysis of variance (ANOVA) of air layered *D. tripetala* seedlings

Source	DF	SS	MS	F-Value	P-Value
Factor	2	1348	6739.90	99.63	0.000
Error	21	1421	67.95		
Total	23	14900			

Table 2. Growth parameters of *D. tripetala* under the interaction effect of top soil and saw dust substrates

Sample	Collar diameter	Height	No. of leaves
A1	$8.1 \pm 0.6$	$60.1 \pm 3.4$	$30 \pm 9.1$
A2	$7.2 \pm 0.4$	$52.0 \pm 3.2$	$7 \pm 1.2$
B1	$11.2 \pm 0.5$	$55.4 \pm 3.8$	No data
B2	$7.2 \pm 0.3$	$59.2 \pm 3.9$	No data
C1	$8.6 \pm 0.4$	$61.2 \pm 5.1$	No data
C2	$7.9 \pm 0.7$	$58.9 \pm 4.5$	$4 \pm 0$
D1	$7.6 \pm 0.7$	$62.5 \pm 7.5$	$33 \pm 11.6$
D2	$6.8 \pm 1.2$	$63.0 \pm 4.0$	$3 \pm 0.0$

Key to samples: A – February, B- March, C- April, D- May  
1-Top soil, 2- saw dust  
(mean  $\pm$  standard error)

Collar diameter was highest in the month of April ( $7.9 \pm 0.7$ ) for marcotted plant from saw dust and least in the month of May ( $6.8 \pm 1.2$  mm). From the result of interaction of saw dust substrate, height had the least mean value in the month of February ( $52.0 \pm 3.2$  mm) and highest mean value in the month of May for marcotted plant from saw dust substrate ( $63.0 \pm 4.0$  mm). Number of leaves was highest in the month February ( $7 \pm 1.2$  mm) and least in the month may for marcotted plant from saw dust ( $3 \pm 0.0$  mm).

*Effect of top soil and saw dust substrate across the growth parameters percentage*

Collar diameter had the highest and lowest percentage 18% and 17% in the months March and May from top soil substrate respectively. Results from saw dust substrate reveals that in the months of February and March both have the same percent value of 12% while it was highest in the month April with percent value of 13% but lowest in May with percent value of 11%.

As showed in Fig. 2, result revealed that there was a decrease in height in month of February (13%) to March (12%) and later stabilised from April to May (13%) in top soil substrate. As for saw dust substrate, highest percent value was evident in month May (14%) and was least in February (11%). There is a constant value from the second and third months which later increased in the last month.

The number of had the highest percentage value of 44% in month of May in top soil substrate and was least in February, as shown in Fig. 1. However, there was a missing value in March and April as leaves were completely absent on the stems. In contrast, percentage value in saw dust substrate was lowest in month of May (4%) and highest in February with a percentage of 39% and there was also a missing percent value in March due to same reason as mentioned above (Fig. 2). The month of March experienced senescence due to physiological behaviour of the seedlings as the matured mother tree.

The effect of the different substrate showed that top soil substrate has a very good performance on the air layered seedlings and also across the growth parameters because the highest percentage value was recorded in top soil substrate.

The research findings from silvicultural study gave an edge as compared to all other related studies that has been carried out so far on aird layering of indigenous fruit trees (IFTs). According to literatures, all studies carried out on layering of IFTs stopped at the rooting stage of the marcotted stems on the mother tree. There was no continuous study as regards to the growth assessment of the severed stem from the mother tree. Vegetative propagation of plants is essentially the reproduction of plant material

from vegetative organs so that the offspring will contain the exact characteristics of the parent plant with regard to genotypes and health status (Macdonald, 1996). Air layering as a vegetative method captures the attributes of elite trees within genetically diverse wild populations to avoid long, slow process of tree breeding (Tchoundjeu *et al.*, 2006) and promotes a high success rate of propagation (Diane and Elizabeth, 2009). With a success rate of 28.33% in this study, the ability of *D. tripetala* to multiply by air layering is very high. This is in contrast to what was obtained when compared to *Detarium microcarpum* with a low success rate (Ricez, 2008). The results of the experiment carried out by Tchoundjeu *et al.* (2010) showed that auxin treatment decreased the rooting percentage and increased the mortality rate of *Irvingia gabonensis* marcotts. Several trials of air layering have however given more satisfactory results on other species: *Pterocarpus erinaceus* Poir. with 100% success (Zouggar 2008), *Balanites aegyptiaca* (L.) Del. with a success rate of 95% (Tchiagam *et al.*, 2011), *Cola edulis* Baill. with 48% success (Moupela *et al.*, 2013), and *Prunus azorica* (Hort. ex Mouillef.) Rivas Mart. with 100% success in Azores (Moreira *et al.*, 2009).

As a result of the physiological maturity cycle of the plant marcotted, senescence was experienced, leading to the loss of leaves in seedlings. This affected the initial rate of photosynthesis, therefore a reduction in the net assimilation rate (NAR). The material in the vascular bundle reserve, developed new roots which assisted in assimilation of nutrients from the soil. New structures are developed and increase in growth of the marcotted seedlings (Fig. 3).

Table 3. Foliar micro-characters of *D. tripetala*

Micro-characters	Abaxial	Adaxial
Stomata type	Anomocytic	Absent
Stomata length	23.2±1.5	Absent
Stomata width	17.6±1.9	Absent
Cell type	Polygonal	Polygonal
Cell length	37.1±8.8	30.4±2.6
Cell width	22.0±5.2	15.4±2.3
Cell wall thickness	1.7±0.4	1.8±0.6
Anticlinal wall pattern	Sinose	Straight – sinose
Druces	Present	Present

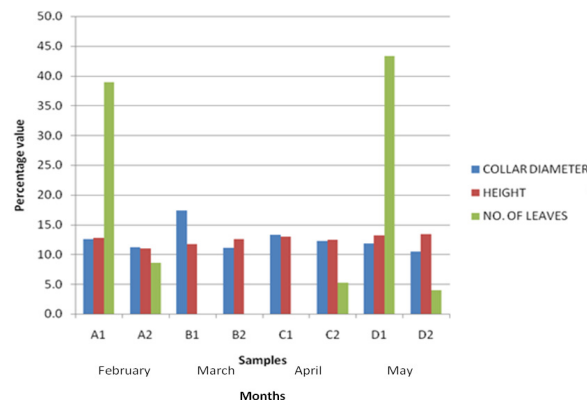


Fig 2. Percentage value of the different substrates across the various growth parameters





Fig. 3. Rooting system in *D. tripetala*. A: Air-layering using saw-dust. B: Air-layering using sterilized sharp sand

Further findings from micro-morphological studies of *D. tripetala* showed that the species is hypostomatic, and has some unique characteristics that may be useful for its identification. Foliar epidermal cells are predominantly polygonal with straight to sinose anticlinal walls (Plate 1). These cells are larger on the abaxial surface than on the adaxial. They measure up to an average of  $37.1 \times 22.0 \mu\text{m}$  and  $30.4 \times 15.4 \mu\text{m}$  in size on the abaxial and adaxial surfaces respectively. Stomata is anomocytic, up to an average of  $32.2 \times 17.6 \mu\text{m}$  in size on the abaxial surface and completely absent on the adaxial (Table 3). Crystals are also present on both surfaces while trichome is entirely absent. As noted by

Peres-Estrada *et al.* (2000), plants growing in sun exposed areas tend to have higher trichome densities than those in shady environment. The absence of trichome in *D. tripetala* may thus be attributed to its habitat. Wood is vesselless, composed only of imperforate tracheary elements and parenchyma; tyloses absent; gums present; fibres thin-thick walled; axial parenchyma rare. Fibre septate; ray multiseriate with some associated uniseriate cells,  $>1 \text{ mm}$  (Plate 2). Pollen grains are in tetrad; acalymmate; prolate rhomboidal in shape. P  $55.0\text{--}(61.0)\text{--}64.4 \mu\text{m}$ ; E  $51.0\text{--}(58.4)\text{--}63.2 \mu\text{m}$ . Exine is generally thin;  $2.0\text{--}3.0 \mu\text{m}$  thick; surface scabrate (Table 4, Plate 3).

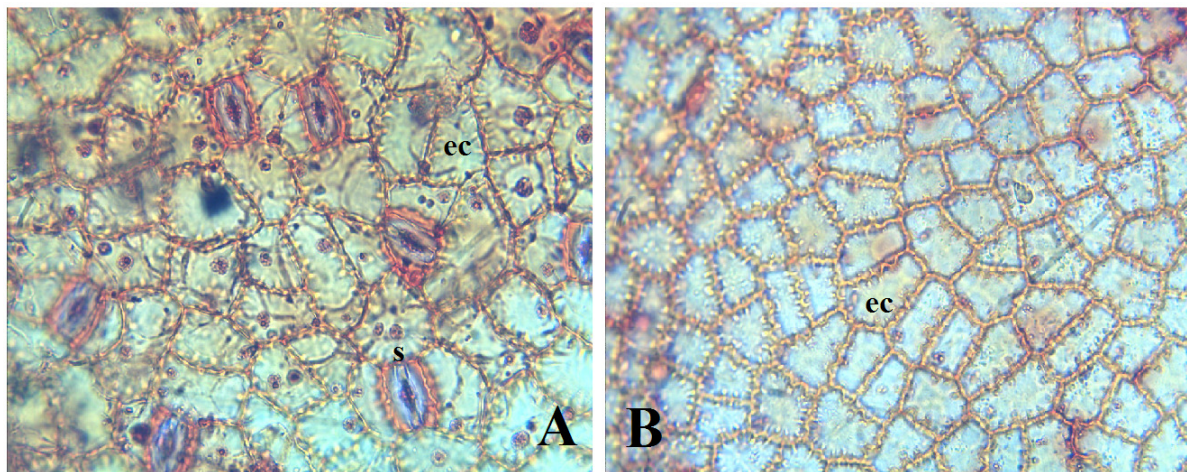


Plate 1. Photomicrographs of the foliar epidermal layers of *D. tripetala*. X400. A: abaxial surface; B: adaxial surface. ec- epidermal cell; s- stoma

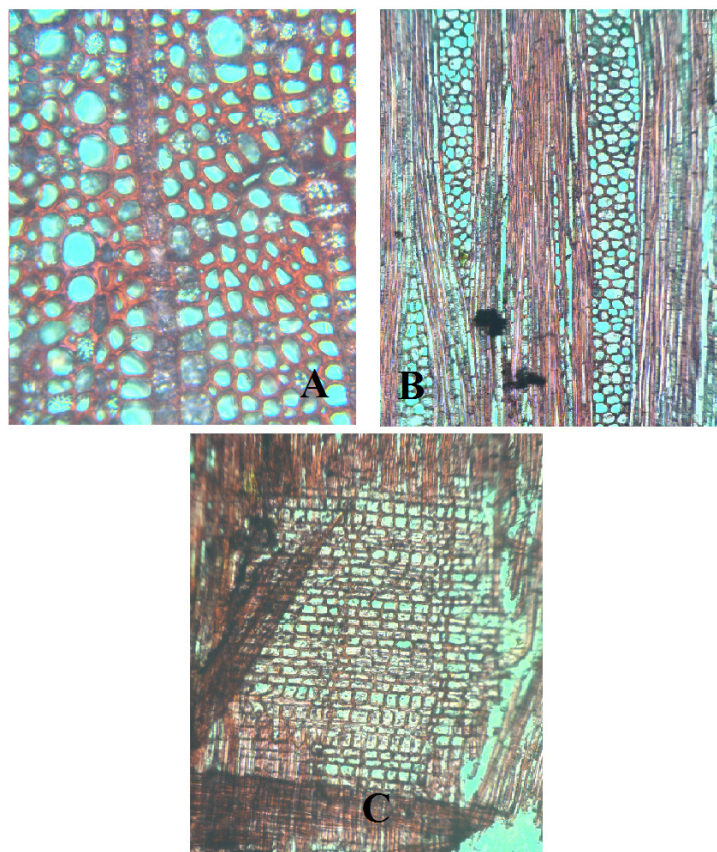


Plate 2. Photomicrographs of the stem of *D. tripetala* x400. A- transverse section; B- transverse longitudinal section; C- radial longitudinal section



Plate 3. Photomicrograph of *D. tripetala* pollen X 400

Table 4. Pollen characteristics of *D. tripetala*

Exine thickness (μm)	2.0-3.0
Polar diameter (μm)	55.0-64.4
Equatorial diameter (μm)	51.0-63.2
Shape	Pro-rhomboidal
Surface	Scabrate

layering. The best growth assessment was obtained from marcotted stem of top soil substrate. The development of rapid and more efficient vegetative propagation techniques for important but neglected indigenous African tropical fruit trees species like the pepper fruit will to a very large extent facilitate the full domestication and fruit production potentials. This would be a very efficient and effective way of combating hunger and by extension, extreme poverty. The present study thus have shown that propagating trees of the species by air layering is possible and this technique can be used to multiply and keep this species, which will reduce the regeneration problem linked to a low seed germination rate and too much demand on the seeds. *Dennettia tripetala* showed a good aptitude for being propagated by air layering. Morphological studies have also revealed that the species has some interesting characteristics such as presence of cell inclusions/crystals on both surfaces of the leaves, complete absence of trichomes, polymorphic nature of wood rays as wells as the unique shape and sizes of

## Conclusions

In the present study, efforts were made to develop an efficient, rapid and inexpensive method for vegetative propagation of *Dennettia tripetala*. The results indicate that it is possible to successfully propagate pepper fruit by air



the pollen grains. Ecological data reported by herbarium collectors showed the spread of this economic species across the forest areas of Nigeria, however, with the continuous degradation of our ecosystems, there is the need for sustainable collection and use of our rich but endangered flora species such as *D. tripetala* to checkmate total disappearance of these important life sustainers.

### Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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