Nursery substrates and provenances influence rooting performance of juvenile, single-node vine cuttings of *Gnetum africanum* Welw. (Gnetaceae)

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*Gnetum africanum* Welw. (Gnetaceae) is a forest vine that is highly valued for its leaves which are a source of food and income. Because wild populations are threatened by over-harvesting, there is an increasing need to cultivate the plant, which in turn entails a need for developing good quality planting material. This study investigates the effects of four provenances, four substrates and their interactions on rooting and shoot development of vine cuttings of *G. africanum* using the non-mist propagation system developed by ICRAF. Single-node, half-leafed vine cuttings were used and data on rooting and leaf production were collected monthly from the second to the fifth months. Rooting percentages were subjected to analysis of variance using logistic regression procedures in Genstat version 12. Provenance (P<0.001), substrate (P<0.001) and interaction between provenance and substrate (P<0.001) showed highly significant effects on rooting. Excel was used to determine rooting percentages, with the highest observed in fine sand on cuttings from Lekie-Assi (81%) and Boumnyebel (79%) which are high *G. africanum* exploitation areas in the Centre Region of Cameroon. Boumnyebel showed a significantly higher shoot development than the other provenances. Fine sand and sharp sand can be recommended for rapid, low-cost production of *G. africanum* planting material.

Key words: domestication, rooting substrate, non-mist propagator, vine cuttings

Introduction

Vegetative propagation of plants is the process whereby an exact copy of the genome of a mother plant is produced. This is made possible because the system uses meristematic, undifferentiated cells that can differentiate into organs necessary to form a whole new plant (Wiesman & Jaenicke, 2002). The classic approach is propagation by stem cuttings, in which roots are induced to form on a piece of stem detached from
a donor plant (Libby, 2004). Several endogenous and exogenous factors such as water and energy status, hormonal balance, mineral and health status of cuttings, age of the cutting, propagation environment and stock plant management influence the success of this process (Wiesman & Jaenicke, 2002). Adequate stock plant management gives the opportunity to enhance the rooting ability of cuttings by providing the appropriate morphological and physiological conditions for shoot development (Leakey, 2004).

_Gnetum africanum_ Welw. (Gnetaceae) is an understorey liana found in the humid tropical forests of Nigeria, Cameroon, Central African Republic, the Republic of Congo, Gabon, Democratic Republic of Congo and Angola (Mialoundama, 1980). Some thirty species of the genus _Gnetum_ occur in the tropics, but only two of these, _G. africanum_ and _G. buchholzianum_, are found in Africa (Mialoundama & Paulet, 1986). In Cameroon, _G. africanum_ is often found in fallow farmlands and secondary and closed forests. The vines climb on trees, saplings and shrubs for support in the complex tropical humid forest where they grow abundantly producing great quantities of leaf biomass (Shiembo et al., 1996). _G. africanum_ has a tap root system, bearing numerous hair roots (Onguene & Kuyper, 2001) and is capable of generating root suckers, as offshoots of lateral adventitious roots (Halle et al., 1978; Nkefor et al., 2000). This suckering can be quite prolific in the wild, suggesting that vegetative regeneration is important (Sunderland, 2001). _Gnetum_ is shade-tolerant and does not do well in full sunlight (Schippers, 2000; Shiembro, 1997). However, _G. africanum_ growth may be favored by forest disturbance, which may explain its abundance in degraded forests, bush fallows and crop fields (Fondoun & Tiki Manga, 2000; Mialoundama, 1993). Ectomycorrhizae (Sceloroderma sinnamariense) have been found in association with cultivated and wild _Gnetum_ plants (Limbe Botanic Garden, 1998; Onguene, 2000) and this symbiotic relationship is believed to enhance the species’ ability to take up nutrients from the soil. According to Bechem and Alexander (2012), plants of _G. africanum_ are almost always colonized by ectomycorrhizae, suggesting that colonization might be a prerequisite for normal growth, development and survival of the young plants. The latter authors further point out that _Gnetum_ species are tropical plants, thriving in environments where there is a seasonal fluctuation of nutrients. Hence, mycorrhization would help the plant to survive such nutrient fluctuations and the competition that thus arises. However, such dependence on one or a few fungi places the plant at risk, as in the absence of this particular inoculum, environmental factors would greatly affect the plants’ establishment and survival (Bechem & Alexander, 2012). Kitchen ash (2400 kg/ha) and poultry manure (2400 kg/ha) have been shown to enable better field establishment, leaf production and survival of _G. africanum_ plants on highly leached and strongly acidic soils (Ultisols), as opposed to single super phosphate (400 kg/ha) and urea (400 kg/ha) in southern Nigeria (Ibeawuchi et al., 2008).

In many forest communities _G. africanum_ is highly valued as a vegetable playing a significant nutritional and social role across the sub-region where it is found and consumed by people of all social strata (Mialoundama, 1993). Leaves are shredded and prepared in soups and eaten with fermented cassava paste locally called “water fufu” in Cameroon.
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It is also added to stews or eaten raw. The nutritive value of *G. africanum* is reported by Mialoundama (1980) to be very high. The leaves present an important source of proteins, essential amino acids and minerals. A chemical analysis identifies the leaves as a good source of sodium, potassium, calcium, magnesium and iron (Okafor et al., 1994).

In Cameroon, leaves of both species are harvested on a daily basis for sale in local and regional markets. Plants are evergreen and therefore leaves are available throughout the year (Shiembo et al., 1996). The leaves are collected either during specific collection trips or opportunistically, in the course of other activities (Henkemans, 1995). A number of sources indicate that currently all *Gnetum* leaves marketed in Cameroon are harvested directly from the wild (Nchinda et al., 2008; Clark and Sunderland, 2004). Actual harvesting of leaves often entails plucking them from the slender vines and side shoots, which if done carefully allows the individual plant to regenerate. According to Shiembo (1997), there are increasing reports of destructive and unsustainable harvesting practices, such as the cutting and removal of entire plants and/or felling of support trees. Moreover, both *Gnetum* types are Red List classified as near-threatened species (Lakeman Fraser & Bachman 2008; Baloch 2009). According to Bokwe and Ngatoum (1994), there is an urgent need to ensure conservation of these species. In 2009, the Ministry of Agriculture and Rural Development of the Republic of Cameroon approved a project to support the domestication of *Gnetum* species.

Most of the *Gnetum* from Cameroon is transported by road to Idenau near Limbe in the South West Region of the country. From there it is exported by boat to Nigeria which is a major market for the resource (Awono et al., 2002). Export markets to the African diaspora in Europe are large, as is commercial activity from Nigeria to the United States of America (Shiembo 1997; Tabuna 1999; Ladipo 1997). According to Nkwatoh et al. (2010), about 610,000 metric tons of *Gnetum* was produced and traded in Cameroon and Nigeria between 2002 and 2008. This was valued at about 630,000,000 F (CFA), which is the equivalent to approximately $1,250,000 (USD) of internally generated revenue (IGR) for the economies of Cameroon and Nigeria. Trade in the leaves of *G. africanum* and *G. buchholzianum* is important within and between the countries of the Congo Basin. These include Cameroon, Gabon, Equatorial Guinea, Congo-Brazzaville, Democratic Republic of Congo, the south of the Central African Republic, and the humid zones of Nigeria (Ladipo 1997; Shiembo 1997; Sunderland and Obama 1999; Yembi 1999).

In addition to being a major source of food and income, *G. africanum* has medicinal properties. Leaves are used in Nigeria for the treatment of enlarged spleen and sore throats, and are also considered as an antidote to some poisons. Leaves are also used as a dressing for warts and boils and as a decoction to reduce pains during child birth in Congo-Brazzaville (Shiembo, 1999).

*G. africanum* can be considered a ‘Cinderella’ species overlooked by science (Leakey and Newton, 1994). It provides nutritional, economic and environmental benefits. Farmers view such species as a natural resource and have not nurtured them. However, the sustainability of the species continues to be threatened by deforestation (Leakey, 1999). To arrest this unfortunate situation, *G. africanum* has been subjected to domestication over the past fifteen years (Tchoundjeu et al., 2006). Seed germination trials
under nursery conditions have not been encouraging as seeds often take up to 7 months to one year to germinate (Okafor, 1997; Shiembo, 1999; Ndam et al., 1997). The rooting of vine cuttings was then resorted to as a low-cost solution to the problem of providing sufficient quantities of improved planting material for on-farm cultivation. In doing so, cuttings from different provenances were set in different substrates to evaluate their effects on the rooting success and shoot development of G. africanum vine cuttings.

Materials and Methods

Study Site

![Figure 1. Map of Cameroon showing provenances of G. africanum vine collection used in this study (Source: Ngaunkam, 2014)](image)

Plant material used in this trial was collected from four locations (Lekie-Assi, Boumnnyebel, Mbalmayo and Mfou) where Gnetum is gathered extensively in the Centre Region of Cameroon for marketing and household consumption (Figure 1). All four provenances are located within the bimodal (two rainy and two dry seasons) rainfall zone with altitudes of between 500-1000 m asl. Lekie-Assi and Boumnyebel have a sub-equatorial climate with mean temperature of 25°C and relative humidity of 75%. The average annual rainfall ranges from 1300-2500 mm while the soils are sandy, sandy clay loam or clay in texture (Ndô et al., 2010). Mbalmayo (640 m above sea level; 3°25N, 11°28E), has a total annual rainfall of about 1500 mm. The soil type is an Ultisol derived from schist band (De Cauver
et al., 1995). The mean annual temperature is approximately 24°C and the soils are deep ferralitic (Ambassa-Kiki, 2000). Mfou (30 69’N, 110 69’E) is located within the equatorial climatic zone. It has a mean annual rainfall of 1530 mm and the soils are acid ultisols (Duindam and Hauser, 2008). Hulugalle and Ndam (1993) however, identify the soils of Mfou as clayey or kaolinitic. The trial was conducted at the ICRAF nursery in Nkolbisson, Yaoundé, Cameroon (altitude: 700m above sea level, latitude 30 52’-30 53’N, and longitude 110 25’-110 27’E). The average annual rainfall is 1692 mm and the rainfall pattern is bimodal. Relative humidity varies generally between 73% and 84%, and the average temperature is 25°C (Ambassa-Kiki, 2002).

Collection of plant material and setting of cuttings

About fifty young vines of G. africanum growing wild in the forest were collected (above the third node from the base) from each provenance, watered and stored in a disinfected, humid polythene bag. They were transported in a closed-back pick-up vehicle to the nursery (a journey of no more than 45 minutes) where vines were watered again using a knapsack sprayer before cuttings were prepared. Cuttings were cut to about 5 cm lengths with circular base to allow for uniform root distribution and slanting upper surface to ease run-off during watering (Tchoundjeu, 1989); whereas leaves were halved to about 50 cm² surface area to reduce transpiration and maintain photosynthesis to allow for cutting survival (Longman, 1993). Each prepared cutting was dropped into a bucket of clean water to prevent water loss. Cuttings were then set on four substrates including fine sand dug from a sand pit (with fine particles of less than 0.2 mm), sharp sand from a river (with coarse particles of about 1-2 mm), decomposed sawdust from an abandoned saw milling site and a 50:50 mixture of decomposed sawdust and sharp sand) which had been treated with fungicide containing dimethoate (50 g/16 litres of water) as active ingredient and insecticide containing cypermethrine (50 ml/16 litres of water) as active ingredient five days before use. The trial was conducted in a non-mist propagator (Leakey et al., 1990) divided into twelve compartments in which the four substrates were randomly allocated. Each compartment was subdivided into four sub-units in which cuttings from the 4 provenances were randomly allocated (Figure 2). Ten cuttings were set per sub-unit, resulting in 120 cuttings per provenance and 120 per substrate making a total of 480 cuttings for the trial. Watering was carried out using plastic tubes with water allowed to flow until it reached the same level as the gravel layer, keeping the overlying substrate moist. The propagator was placed in a shade house which has alternating rows of translucent and corrugated iron roofing sheets (Longman, 1993). It is bordered with shade cloth allowing about 70 percent of irradiance to enter the propagator.
Observations and measurements

The propagator was opened daily in the morning to verify the water level, which had to be at the same level as the gravel layer for adjustment, a check was carried out for fallen leaves which were removed to avoid microbial attack from decaying leaves. The inner surface of the transparent polythene sheet covering the propagator was cleared of mist which had settled on it. To minimize water loss, cuttings were watered using a knapsack sprayer whenever the propagator was opened. From the second month after setting, measurements were taken once a month for four months. Each cutting was lifted from the substrate and examined for the presence of roots. A cutting was considered as having rooted if there was one or more roots that were at least 1 cm in length (Atangana et al., 2006). Rooted cuttings were assessed for number of main roots (primary roots originating from cutting base), length of longest root, number of new leaves, number of new vines and vine length. Rooted cuttings were then transplanted into polythene bags containing forest soil and sharp sand (2:1) to ease percolation after watering. Forest soil was collected from a Gnetum stand in Lekie-Assi (within 15 to 25 cm below ground) and supposed to contain mycorrhiza which enhances nutrient uptake and growth of G. africanum. Cuttings that had not rooted were put back into the substrate.
**Data analysis**

The rooting percentage in each treatment was assessed using graphical tools in Excel 2007. To determine the effect of experimental factors on rooting ability and shoot development of cuttings, data were subjected to analysis of variance using Generalized Linear Model procedures in Genstat version 12 software. Effects of provenance, substrate and their interactions on the percentage of rooted cuttings were assessed using the logistic regression model (binary data), while a log linear regression model was fitted to the root number data following the Poisson distribution. Factors having a significant effect on cutting rooting ability were compared among treatments using the Least Significant Difference (LSD) procedure considering confidence interval of 0.05.

**Results**

The results of analysis of variance showed that time ($P=0.004$), provenance ($P<0.001$), substrate ($P<0.001$) and the interaction between provenance and substrate ($P<0.001$) had significant effects on rooting.

![Figure 3. Rooting percentage (%) of 4 provenances of G. africanum cuttings in 4 nursery substrates](image)

Cuttings from Lekie-Assi on fine sand had the highest rooting rate followed by those from Boumnyebel, also on fine sand, but there was no significant difference between them. Rooting rates of cuttings from these two provenances were significantly higher than the Mbalmayo rooting rate, which was also significantly higher than the rooting rate for cuttings from Mfou. Cuttings from Lekie-Assi showed a significantly higher rate of rooting on sharp sand, compared to those from Boumnyebel, which was also significantly higher.
than those from Mbalmayo and Mfou, between which there was no significant difference. For the mixture of sawdust and sharp sand, cuttings from Lekie-Assi had the highest rooting percentage, followed by cuttings from Boumnyebel that had a significantly lower rate of root production, which was, however, significantly higher than root production on cuttings from Mbalmayo and Mfou, between which there was no significant difference. The highest rate of rooting on sawdust was observed on cuttings from Boumnyebel, which had a significantly higher rate than that of cuttings from Lekie-Assi (Figure 3). In terms of number of roots per rooted cutting, Figure 4 shows that fine sand induced significantly higher numbers of roots on cuttings regardless of provenance, compared to the other substrates. This was followed by sharp sand, mixture and sawdust. On the other hand, the number of roots produced per cutting from different provenances showed that cuttings from Lekie-Assi produced significantly more roots followed by those from Boumnyebel, Mbalmayo and Mfou.

Figure 4. Root production of G. africanum vine cuttings from Lekie-Assi on different substrates at 2 months after setting

Figure 5. Root production of G. africanum vine cuttings from different provenances in fine sand at 2 months after setting
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Figure 6. Effects of four provenances on growth characteristics of rooted cuttings of G. africanum

Cuttings from Lekie-Assi produced a significantly higher number of main roots than cuttings from Boumnyebel and Mbalmayo, between which there was no significant difference, whereas both produced significantly higher numbers of main roots than cuttings from Mfou. Cuttings from Boumnyebel produced significantly longer and higher numbers of vines and leaves respectively, than those from Lekie-Assi, Mbalmayo and Mfou, which showed no significant differences for these growth characteristics. Roots produced on cuttings from Lekie-Assi were significantly longer than those on cuttings from the other three provenances, between which there was no significant difference as shown in Figure 6.
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**Figure 7. Effects of four substrates on growth characteristics of rooted cuttings of G. africanum**

Fine sand allowed the production of a significantly higher number of main roots than sharp sand, which in turn induced a significantly higher number of main roots than sawdust and the mixture of sharp sand and sawdust. No significant difference was observed in the number of vines, number of leaves and vine length of cuttings on any of the four substrates. Roots produced on fine sand were significantly longer than those produced on sharp sand, which were also significantly longer than those produced on sawdust and the mixture of sharp sand and sawdust as shown in Figure 7.

**Discussion**

This study revealed that fine sand was the best substrate for inducing the rooting of *G. africanum* cuttings among the four substrates tested. Lekie-Assi was the best of the four provenances used. The findings of this investigation are in line with those of Caspa et al. (2009) and Mesen et al. (1997) who found that fine sand induces better rooting on juvenile stem cuttings of *Nauclea diderrichii* and *Cordia alliodora*, respectively, than sawdust. Opposed to this, Ndam et al. (1997) recommended a mixture of 50% sawdust and 50% sand, while Shiembo (1997) suggested the use of 100% decomposed sawdust in a propagator for rooting *G. africanum* vine cuttings.

It is probable that fine sand, and to an extent, sharp sand have the properties of an adequate rooting substrate as described by Anderson (1986) as one with an optimal volume of gas-filled pore space and an oxygen diffusion rate that allows proper respiration. Contrasting reports from different authors on the rooting of *G. africanum* could be due to
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differences specific to the plant materials used. From the top of a plant to the bottom, there is a within-shoot gradient in age that affects the leaf size, leaf water potential, leaf carbon balance, leaf senescence, internode length, internode diameter, stem lignification, nutrient and stem carbohydrate content and respiration.

The gradients in some of these factors mean that no two cuttings are physiologically identical and hence no two cuttings have the same rooting capacity (Leakey, 2004), the substrate notwithstanding. The latter author points out that the same species propagated under relatively similar conditions by different persons can produce results that appear to be contradictory, while in fact their results are expressions of the different physiological and morphological conditions of the tissues being propagated. Stock plants used by the cited authors grew in different environments with varying soil and climatic conditions which can affect their morphology and physiology (Leakey, 2004), and possibly the rooting abilities of cuttings obtained from them. This is further illustrated by Tchoundjeu and Leakey (2000) who found that relative concentrations of carbohydrates and nutrients in cutting tissues as stored reserves for successful rooting vary between node positions and over time in Khaya ivorensis.

Hartman et al. (1990) recognized the importance of using the “right” propagation substrate for optimal rooting of leafy cuttings, while Loach (1986) suggested that substrates with relatively high water content like sawdust are generally associated with higher rates of water uptake in the cuttings and consequently higher rooting percentages. However, the latter author also warns that water can present a major diffusion barrier to oxygen so that excess water may result in anoxia at the cutting base. In this trial, some cuttings that had been set on sawdust and to a smaller extent on the mixture of sharp sand and sawdust showed signs of rotting at cutting base starting one month after setting. When rot occurred, this was followed by leaf yellowing, leaf fall and complete death of affected cuttings. The number of cuttings with these symptoms increased with time. The reason for this is not clear since the water level was controlled through daily observation of water level and the substrate disinfected with fungicide (dimethoate (50 g/16 litres of water)) and insecticide (cypermethrine (50 ml/16 litres of water)) before cuttings were set. The substrates in this trial were moist, but not too wet. However, it is possible that sawdust and the mixture of sharp sand and sawdust retained most of the water that was taken up, resulting in the rotting of some cuttings. The reason for better rooting of cuttings from Lekie-Assi and Boumnyebel could be genetic or possibly due to juvenility of plant material from which cuttings were collected. From field observations, these populations underwent more active exploitation; so the material sourced here was possibly relatively young compared to those from Mbalmayo and Mfou which only experienced occasional exploitation. It is also possible that better performance of growth indicators observed on cuttings from Boumnyebel is due to juvenility of plant material used.

**Conclusion and Recommendations**

The results of this study indicate that G. africanum can be propagated vegetatively from leafy vine cuttings with fine sand and sharp sand as rooting substrates in a non-mist propagator. Cuttings from Lekie-Assi and Boumnyebel showed better rooting than
those from Mbalmayo and Mfou. Fine sand recorded a rooting success of up to 80% for cuttings from Lekie-Assi and Boumnyebel, followed by sharp sand with 60%. With the dwindling population of Gnetum in the wild and the increasing need for its domestication to meet high demand, farmers can depend on this low-cost technology using fine sand and sharp sand to produce improved planting material in adequate quantities for on-farm planting operations. As a follow up to this study, it will be necessary to investigate the effects of cutting age/position and mixtures of substrates in different proportions in combination with other factors such as cutting length and provenance to optimize the rooting performance of G. africanum.

References


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