Vegetative propagation of *Garcinia lucida Vesque* (Clusiaceae) using leafy stem cuttings and grafting

Bertin Takoutsing, Alain Tsobeng, Zacharie Tchoundjeu, Ann Degrande, Ebenezar Asaah

World Agroforestry Centre (ICRAF), Cameroon

Garcinia lucida Vesque (Clusiaceae) is a tree species that is highly valued for its medicinal properties by rural households in the humid forest zone of Cameroon. However, the unsustainable exploitation of the species threatens its long-term regeneration. This study focuses on its vegetative propagation via stem cuttings in non-mist propagators and through grafting. The study tests the effects of three rooting media (sand, sawdust, sand + sawdust (1/1); three leaf sizes (0,25 and 50 cm²); and three types of hormone [indole butyric acid (IBA), indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA)], applied as a single dose. Furthermore, three grafting techniques (cleft, side tongue and whip-and-tongue grafting) were tested. All experiments were designed as completely randomized blocks with three replicates. Results showed that cuttings require a medium that has low water holding capacity and high porosity, and a leaf area of 50 cm² and NAA treatment; grafting success was affected by the technique used, with top cleft grafting yielding a 100% success rate. From this preliminary study, it is concluded that *G. lucida* is amenable to vegetative propagation by cuttings and grafting.

Key words: tree domestication, leaf area, non-mist propagator, rooting hormone, rooting medium, under-utilised species

Introduction

Garcinia lucida Vesque (Clusiaceae family) is a small, evergreen dioecious tree of the humid forest zone of west and central Africa, reaching 25-30 cm in trunk diameter at breast height (dbh) and 12-15 m in height in high-density stands. The trees provide a variety of non-timber forest products of great importance to rural households (Guedje and Fankap, 2002). Trees flower and produce fruits throughout the year and seeds germinate within a few weeks of falling. *G. lucida* is found in undisturbed or mature forests at altitudes above 500 m in Cameroon, Equatorial Guinea and Gabon where conditions such as a high annual rainfall (1600-1800 mm) and a mild average daily temperature (24-26°C) favour its growth and development (Bamps, 1970).

The species is highly valued for its medicinal and nutritional properties (Omode *et al.*, 1995). The seeds and bark, either dried or fresh, are widely used to relieve stomach and gynaecological pains (Sunderland and Obama, 1999; Van Dijk, 1999). In addition, the stem bark is traditionally used by forest dwellers in the fermentation of traditional alcohol obtained from palm or raffia trees (Guedje, 2003; Momo *et al.*, 2011).

The most popular method of harvesting the bark is to strip it from the full circumference of the stem. This practice is highly destructive and has been found to be responsible for 74% of tree deaths among harvested trees (Guedje and Nkongmeneck, 2001). This is having serious consequences for the species' regeneration. Tree mortality can be reduced by stripping bark from only one to two thirds of the circumference of the tree's stem. This allows the bark to regrow within about five years (Guedje and Fankap, 2002; Delvaux *et al.*, 2009).

Despite its socio-economic, medicinal and cultural importance, scant attention has been paid to *G. lucida* in terms of research and development. Little is known about its biology and there have been few attempts to improve the tree species, promote its cultivation and/or increase its production. A previous study found that the tree´s germination rate was very high (70-95%) (Guedje and Nkonmeneck, 1999). However, the high demand for seeds by traditional practitioners, coupled with the susceptibility of seeds to pests and diseases and their consumption by wild animals, limits the number of seeds available for germination. As a result, the regeneration of the species is limited.

In general, successful propagation cuttings and grafting depends on both genetic characteristics inherent to the species, as well as the environmental conditions in the nurseries (Leakey, 2004). This preliminary study was designed to determine the optimum conditions for propagation via leafy stem cuttings and grafting, in order to provide useful information on the species for domestication programmes.

Like most tropical trees, *G. lucida* has an allogamous reproductive system: the progeny is genetically distinct from the mother tree. This is a major constraint to domestication by breeding and generative propagation (Caspa *et al.*, 2009), but a benefit for the development of phenotypically superior cultivars by vegetative propagation (Akinifesi *et al.*, 2008). Vegetative propagation ensures that all plants within a cultivar are genetically identical (i.e. true-to-type planting materials) (Meunier *et al.*, 2006; Caspa *et al.*, 2009). Rooting of leafy stem cuttings and grafting have been tested successfully on some priority species at both research station and farm levels. This study on *G. lucida* specifically investigates the effects on the rooting ability of leafy cuttings of three types of rooting media, three auxin types and three different leaf areas, and then tests three grafting techniques.

Materials and methods

Study area

This research was carried out from April to August 2011 at the World Agroforestry Centre (ICRAF) research nursery in Yaoundé, Cameroon (3°52'N, 11°26'E). The site is located in the low altitude, semi-deciduous forest zone where the rainfall pattern is bimodal with 4 distinct seasons, 2 dry seasons (from November to March and in July), and two rainy seasons (from March to June and from August to October). Mean annual rainfall varies between 1600-1800 mm. Mean monthly temperature varies between 23 and 26°C, whereas mean relative humidity ranges between 73-84% (Ambassa Kiki, 2000). The cuttings and scions used in the study were collected at a field situated approximately 40 km from ICRAF's research nursery close enough to have similar weather characteristics as the nursery site in Yaoundé. The rootstocks used in the grafting experiment were produced at ICRAF's research nursery.

Collection and preparation of cuttings

Leafy stem cuttings were randomly harvested from seedlings in natural stands. Prior to taking cuttings seedlings were sprayed with water early in the morning. All tools (pruners, knives) used were disinfected before use. Materials harvested were conserved in a disinfected humid polythene bag: internally black and externally white to minimize heat stress. In the nursery, the leaves of the cuttings were trimmed to yield a surface area of 0, 25 and 50 cm^2. Cuttings were cut to a length of about 4 cm. The single node cuttings had a circular base to guarantee homogenous root distribution and a slantwise upper part to facilitate the runoff of water during watering (Tchoundjeu, 1989).

After this preparation, the basal end of each cutting was dipped briefly into 10 µl of a hormone solution prepared by mixing 50 mg of auxin and 10 ml of 95% ethanol (Avana, 2006). To minimize stress, cuttings were placed in a non-mist propagator following the procedure described by Leakey *et al.* (1990) as soon as they were prepared. The propagators were located in a shade house, allowing about 70% of ambient light to penetrate, in order to maintain constant climatic conditions and so minimize heat stress. Rooting media were treated 7 days prior to the beginning of the experiment with fungicide (Ridomil$^{+®}$, with mefenoxam and copper oxychloride as active ingredients produced by Syngenta; 50g/16 liters of water) and insecticide (Cyperdim 220 EC, with cypermethrin + dimethoate as active ingredients, at 50 ml/16 liters of water) and were kept closed for two days and opened for two days to maximize impact and then limit the risk of toxicity. Each cutting was placed in the propagator such that the medium was firmly placed around it. Cuttings in propagators were sprayed with a fine jet of water whenever the propagator lid was opened for inspection.

Experimental design

Effect of type of hormone on the rooting ability of cuttings

In addition to the control that received only water, three different auxins were tested as rooting hormones. They were indol butyric acid (IBA), indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) at a single dose/concentration of 10 µl l of each of the hormones as previously done by Avana (2006). The experiment was laid out as a completely randomized block with three replications. The rooting medium used in the experiment was washed river sand and each cutting had a leaf area of 50 cm^2. Sixty-three cuttings were set for each treatment. These were allocated to three replicates (n = 252 cuttings).

Effect of rooting medium on the rooting ability of cuttings

Three rooting media (sand, sawdust and a mixture at a ratio of 1:1 of sand and sawdust) were evaluated and the experiment was arranged as a completely randomized block. No hormone was used in the experiment and each cutting had a leaf area of 50 cm^2. Sixty-three cuttings were set for each treatment. These were allocated to three replicates (n = 189 cuttings).

Effect of leaf area on the rooting ability of cuttings

To investigate the effect of leaf area, the experiment was laid out as a completely randomized block with three treatments (0 cm^2, 25 cm^2 and 50 cm^2). River sand was used as the only rooting media and no hormone was used. Sixty-three cuttings were set for each treatment allocated to three replicates (n = 189 cuttings).

Management of the experiments and observations

Every morning, the water level in the propagator was checked and adjusted accordingly, while the transparent plastic was cleaned and the cuttings sprayed with water using a knapsack sprayer. Cuttings were assessed every two weeks for a period of 18 weeks. During each assessment, each cutting was lifted from the rooting media and rooting status noted ("1" for rooted or "0" for unrooted). Dead (rotted) cuttings were recorded ("1" for dead cutting or "0" for alive). Live, but unrooted cuttings were re-set in the media for subsequent observations. During each assessment, the number of roots per rooted cuttings was counted. The cutting was said to be rooted when it had more than one root exceeding 1 cm in length. Rooted cuttings were removed from the propagators and potted into perforated black polythene pots containing a 2:1 mixture of top soil and sand.

Effect of grafting technique on the propagation of G. lucida

Six-month old seedlings produced in the ICRAF nursery were used as rootstocks for the grafting experiments. All rootstocks were 60 cm long and had a collar diameter of about 1 cm. Scions used were collected from young and healthy branches of mature trees with known fruit characteristics located in multistrata secondary forests. Each scion was cut to a length of 20 cm with a diameter close to that of the rootstock.

After collecting the scions, the leaves were trimmed and the material placed in disinfected polythene bags for transportation to the nursery. In the nursery, scions were soaked in a solution of fungicide (Ridomil+®, with mefenoxam and copper oxychloride as active ingredients; 50g/16 liters of water) and placed in the propagator. Under the shade, different types of slits, corresponding to three different grafting techniques (top cleft, side tongue and whip-and-tongue grafting), were made in the stem of the rootstocks at about 20 cm above the collar. Scions were then inserted into these slits, tied and covered with transparent plastic. Grafted plants were then kept in the shade and monitored until the new leaves were completely green, after which they were exposed to direct sunlight for acclimation. All grafting was done by the same person to reduce experimental error.

The experiment was laid out as a randomized complete block design with each grafted plant considered as an experimental unit. Fifteen plants were used per treatment giving a total of forty-five plants. Every week, each grafted plant was assessed to register the growth of the scion ("0" for no growth and "1" for sprouting bud).

Statistical analysis

To determine the effect of experimental factors on grafting and rooting ability of cuttings, percentages of grafted plants budded and dead, percentage of cuttings rooted and dead, and mean number of roots developed per cutting collected were analysed using Linear Regression Model procedures of Genstat V.13 software. Specifically, percentage of grafted plants budded and dead, and cuttings rooted and dead were assessed using Logistic Regression while an unbalanced ANOVA model was fitted to the mean number of roots per rooted cutting. Factors having a significant effect were compared among treatment levels using the Least Significant Difference (LSD) procedure, considering a confidence interval of 0.05.

Results

Effect of type of hormone on the rooting ability of cuttings (Experiment 1)

Auxin-treated *G. lucida* cuttings rooted earlier (weeks 6 and 7) than the control (week 12) resulted in significantly greater rooting percentages (Figure 1) at week 18 ($P<0.001$). Auxin treatment also significantly affected the mortality rate of cuttings ($P = 0.003$). The treatment with NAA had the highest mortality rate, IBA the lowest while IAA was intermediate (Figure 2). The control had the lowest rate of mortality, a rate similar to that of IBA. The type of hormone did not significantly affect the mean number of roots per rooted cutting ($P = 0.498$), but few roots were produced per cutting (1.0 ± 0.41 for control against 1.62 ± 0.28 for IBA).

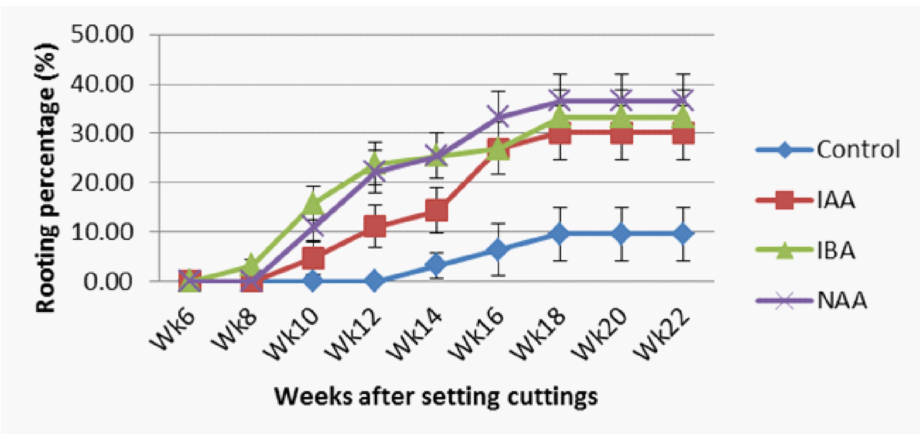

Figure 1. Effect of type of auxin on the rooting ability of cuttings of G. lucida

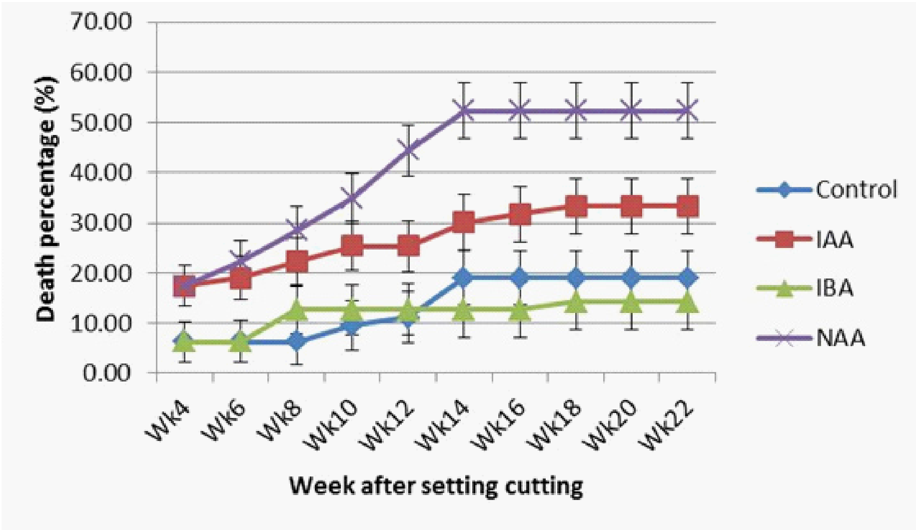

Figure 2. Effect of auxins on the mortality rate of cuttings of G. lucida

Effect of rooting media on the rooting ability of cuttings (Experiment 2)

The percentage of rooted cuttings set in sand was significantly ($P<0.0001$) higher than that of those set in sawdust and a mixture of sawdust and sand (1:1), but had only reached 27% by week 16. Adding sand to sawdust did not improve rooting. Rooting started at week 6 with sand, and at week 8 in sand + sawdust and sawdust (Figure 3). Cutting mortality was significantly affected by the rooting media ($P = 0.003$). Mortality started at 8 weeks for the three rooting media. It was greatest in sawdust (67%) and lowest (37%) in sand (Figure 4).

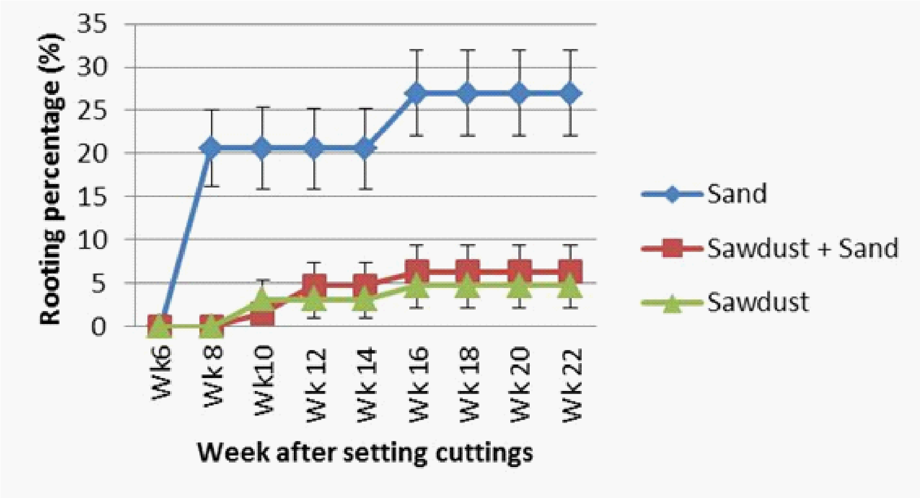

Figure 3. Effect of rooting media on the rooting ability of G. lucida cuttings, without auxin

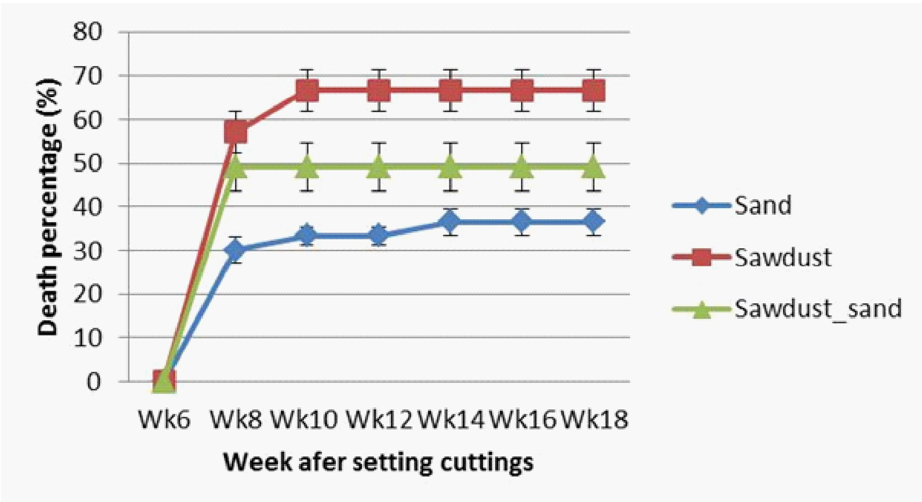

Figure 4. Effect of rooting media on the mortality of G. lucida cuttings without auxin

Effect of leaf area on the rooting ability of cuttings (Experiment 3)

Logical regression demonstrated the highly significant ($P<0.001$) effect of leaf area on the rooting ability of cuttings at week 18. The latency period was evidenced to be 8 weeks. It can be observed that the rooting percentage increased with leaf area (Figure 5) and that leafless cuttings did not root at all. Despite the fact that the highest percentage of rooting cuttings was observed with a leaf area of 50 cm^2, the difference with the 25 cm^2 treatment was not significant. It can further be observed that the mortality rate was significantly ($P<0.001$) higher with leafless cuttings followed by the area 25 and 50 cm^2 leaf area cuttings, respectively (Figure 6). The highest mortality rate occurred between week 6 and 8. On the other hand, no significant difference ($P = 0.62$) was observed for the number of roots per rooted cutting at week 18. The number of roots was 1 ± 0.32 and 1.21 ± 0.29 for 25 and 50 cm^2, respectively.

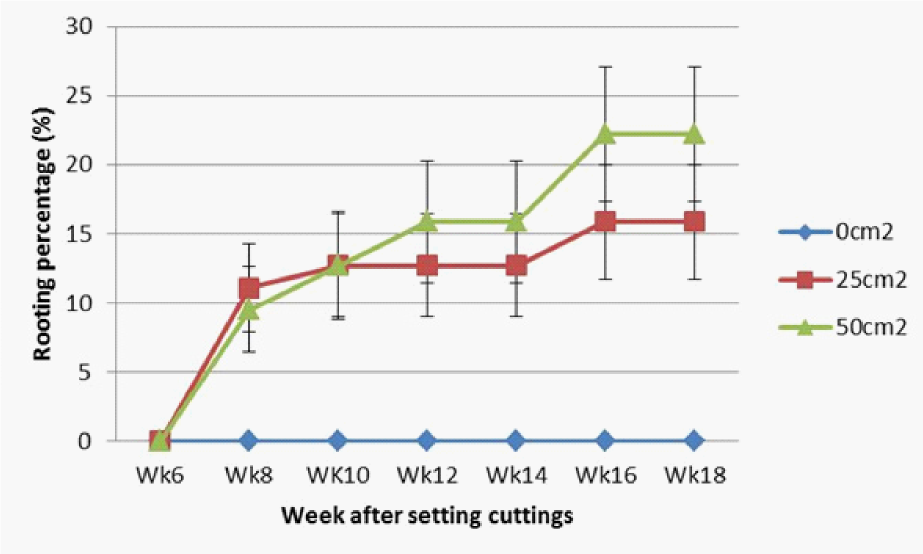

Figure 5. Effect of leaf area on the rooting ability of cuttings of G. lucida

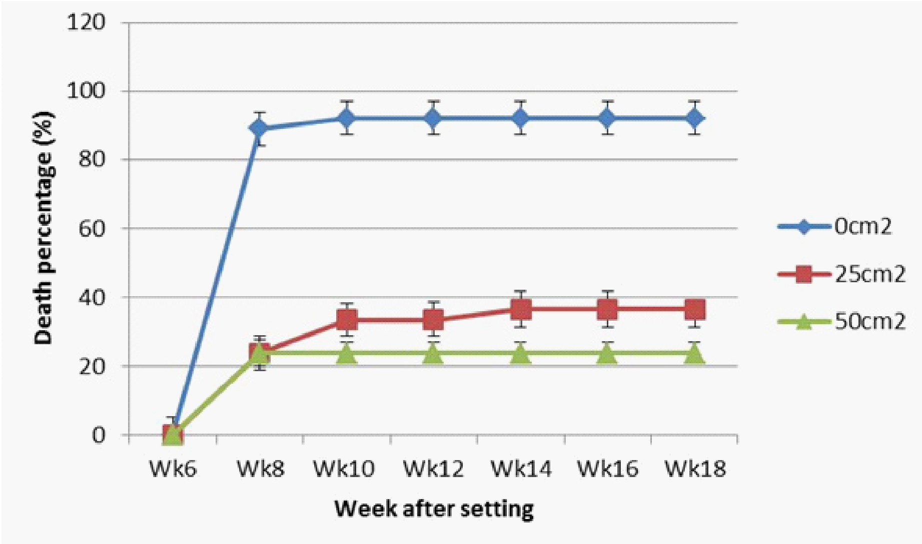

Figure 6. Effect of leaf area on the mortality of cuttings of G. lucida

Effect of grafting techniques on the propagation of G. lucida (Experiment 4)

Acceptable success rates (64–100%) were observed in *G. lucida* with all grafting techniques tested. However, there were significant differences between the techniques (P = 0.012). The best results were obtained with top cleft (100%) and side grafting (90%) (Figure 7).

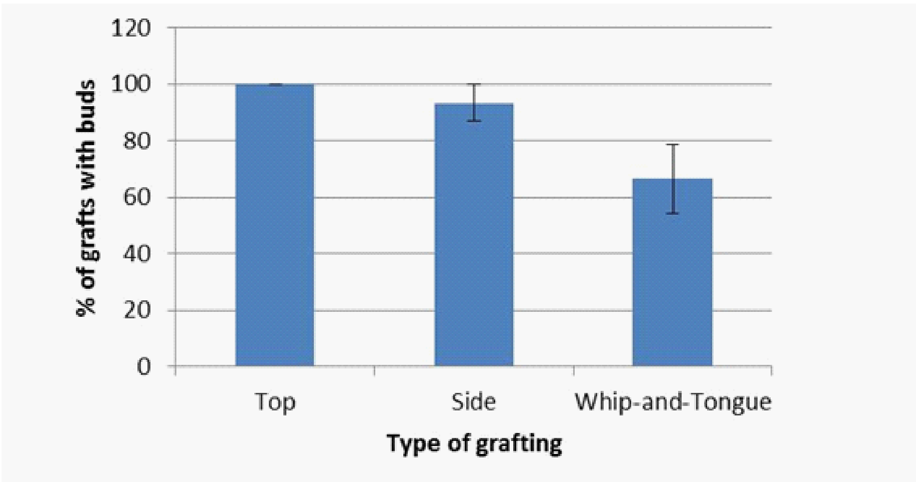

Figure 7. Effect of different grafting techniques on the propagation of G. lucida

Discussion

Effect of type of hormone on the rooting ability of cuttings (Experiment 1)

The application of root-promoting growth regulatory substances (auxins) to the base of cuttings was found to promote the development of roots, as has been documented for many other species (Hartmann *et al.*, 1997). In addition to their effects on cell differentiation, auxins have been found to promote adventitious root formation (Davis, 1988) as well as starch hydrolysis and the mobilization of sugars and nutrients to the cutting base (Das and Mukherjee, 1997; Das *et al.*, 1997). The results of this study agree with findings of studies carried out on other West African trees, such as *Prunus africana* (Tchoundjeu *et al.*, 2002), and *Pausinytalia johimbe* (Ngo-Mpeck *et al.*, 2003). However, behind this apparently 'cure-all' treatment there lies a considerable body of evidence showing that auxin applications interact with other treatments (Palanisamy and Kumar, 1997; Leakey, 2004), types of material (Brennan and Mudge, 1998), and environmental variables (Fett-Neto *et al.*, 2001) in the achievement of eventual rooting success of cuttings. This high degree of interaction is probably why there are many instances in the literature of apparently contradictory statements about the precise physiological role of auxins in the rooting process, a situation that cannot be resolved when authors do not present definitive information about either the physiological condition of their material or the propagation environment used (Leakey, 2004).

Generally, cuttings treated with auxins root more rapidly and produce more roots and usually with a higher percentage of cuttings rooted (Leakey, 2004). Indole-3-butyric acid (IBA) has been found to be the most effective root-promoting auxin, but occasionally α-naphalene acetic acid (NAA) has also been cited as effective; it has been successfully used for the rooting of *Parkia biglobosa* cuttings (Teklehaimanot *et al.*, 1996). However, tree species and even clones can respond differently to auxin applications at differing concentrations, even when other factors are kept constant. Interestingly, clones of *Triplochiton scleroxylon*, which appeared to have different dose response curves, all rooted equally well at 40 µg auxin per cutting (Leakey *et al.*, 1982).

In our study, we did not find any significant difference between the types of hormone applied. While the difference observed may be attributed to auxin concentrations, experience suggests that there are genetic differences between species (e.g. Hartmann *et al.*, 1997; Puri and Swamy, 1999), which could result in differences in rooting ability. Some authors reported that the factors explaining most of the variance are cutting length, leaf area, etc., whereas genetic differences between clones, explain relatively little of variance (Dick *et al.*, 1999). In addition, auxins have different chemical formulae which may act differently in different organisms.

Effect of rooting media on the rooting ability of cuttings (Experiment 2)

This study demonstrated that the best rooting medium for *G. lucida* when not treated with rooting hormone was sand, while the worst was sawdust (1:1). Most of the cuttings that did not root were dead, suggesting that the conditions of this experiment were not optimal. These observations indicate that though the conditions provided by sand were the best, the cuttings suffered from physiological stress. The relationship between rooting percentage and substrate is not as clearly defined as the relationship between substrate and root elongation. According to Kengue (2002), a good substrate should have low water holding capacity (optimum organic matter content) and have a considerable level of porosity (optimum percentage of sand). Therefore, the positive results obtained in this study may be attributed to the high porosity and low water holding capacity of the medium, as also observed by Takoutsing *et al.* (2013). These results also corroborate those of Atangana *et al.* (2006) who found that a suitable substrate for the rooting of *Allanblackia floribunda* can be obtained through modulating porosity by adding sand. Other authors suggested that a good substrate should contain adequate organic matter levels. In this line, a substrate blended with a good proportion of sawdust was successfully used to obtain satisfactory results for *Ricinodendron heudelotii* (Shiembo *et al.*, 1997) and *Diopyros crassiflora* (Tsobeng, 2011). The high mortality rate observed in this study in relation to the use of sawdust could be attributed to the fact that the roots of *G. lucida* are more susceptible to rotting which is promoted by water-logging and anoxia.

Effect of leaf area on the rooting ability of cutting (Experiment 3)

Generally, the rooting of cuttings depends on the presence of a leaf, and indeed on a number of physiological processes of the leaf such as photosynthesis and transpiration. Cuttings without a leaf are expected to quickly become moribund. The most common reason for cuttings failing to root is the death of the leaf due to rotting, necrosis, bleaching or leaf abscission (Leakey, 2004).

All these causes of failure are due to either the use of inappropriate tissues (e.g. photosynthetically inactive), or to an inadequate rooting environment (too hot, too wet or too dry) (Leakey, 2004).

Previous studies have demonstrated the importance of leaf on the cuttings' rooting ability. Rooting ability has been found to be maximized when the severed cutting is photosynthetically active and producing assimilates for the development and elongation of the root primordia, and when the leaf is not suffering from drought stress (Mesén *et al.*, 2001; Leakey, 2004). Consequently, there is an adequate leaf surface area at which the processes of photosynthesis and transpiration are optimal for the survival of the cuttings (Aminah *et al.*, 1997). This would definitely vary between species and clones, depending on specific leaf area (leaf thickness), stomatal density, leaf morphology (waxiness, etc.) and the age of the leaf (Leakey *et al.*, 2004).

Carbohydrate content and hence dry matter, rapidly decrease in cuttings if leaf area is too small, and increase if leaf area is adequate, at least until roots start to develop (Leakey and Coutts, 1989). In our study, leafless cuttings of *G. lucida* could not survive probably due to the rapid use of reserves and slow reconstitution by cuttings. Cuttings with an excessively large leaf area may suffer from transpiratory water loss and subsequent drought stress, and close their stomata, thereby limiting their capacity to photosynthesize and often triggering leaf abscission (Leakey, 2004).

Effect of grafting techniques on the propagation of G. lucida (Experiment 4)

The success rates of grafted plants decrease as the position of the graft on the stem moves from the top to the lowest part of the rootstocks. This might be explained by the gradient physiology in secondary thickening and lignification, the depth of correlative inhibition in buds and gradients in light (shade), humidity and temperature. All these factors may in one way or the other play a part in the success rate of grafting and further studies are needed to determine which are the most important. For instance, Asaah *et al.* (2012) found side tongue grafting to be the most appropriate grafting method for *Allanblackia floribunda*.

Conclusion

This study provides preliminary results indicating the amenability of *G. lucida* to vegetative propagation both for the capture of intraspecific variation in fruit characteristics by grafting, and the subsequent multiplication of a cultivar by leafy stem cuttings. The results obtained for the rooting of leafy stem cuttings of *G. lucida* (50%) are not as strong as those previously obtained by other authors on other West African indigenous fruit species. For example, Mialoundama *et al.* (2002) obtained 80% with *Dacryodes edulis*, Shiembo *et al.* (1997) obtained 80% with *Ricinodendron heudelotii* and Ngo-Mpeck *et al.* (2003) obtained 90% with *Pausinystalia johimbe*. There is clearly a need to improve the rooting success in stem cuttings. Our results suggest that *G. lucida* cuttings respond to auxins, and that both leaf area and rooting medium are important factors that should be optimized. The grafting results were very satisfactory as a success rate of 64-100% was obtained, with the highest value occurring with top cleft grafting.

Other aspects of the propagation environment, as well as pre- and post-severance factors that influence rooting percentage should be examined further: auxin concentration, stockplant management and environment, especially light and nutrient interactions. This will enhance the multiplication of the species through vegetative propagation and solve the problem of seed availability.

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