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Domestication of *Allanblackia floribunda*: Amenability to vegetative propagation

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Abstract

Allanblackia floribunda is a wild and undomesticated forest tree species valued for its nuts used in the food and cosmetic industry. To initiate domestication in the species, the amenability of *A. floribunda* to vegetative propagation was examined through the rooting of single-node leafy stem cuttings using non-mist propagators in Cameroon. Sixty-four cuttings from each of four tested clones (MB, NG1, NG2, NG4) were set in each of four replicate blocks arranged in a randomised split-split plot factorial design. Each main plot was randomly divided into two types of rooting medium (sand or a 50:50 mixture of sand/sawdust). Sub-plots received three types of auxin at 50 μg per cutting (IAA, IBA or NAA) dissolved in 10 μl of alcohol, the control receiving 10 μl of alcohol. Sub-sub plots had four cutting leaf areas (0, 12.5, 25 and 50 cm^2). Interaction of these factors was also investigated. Clone × substrate (variance = 0.01706) and clone × substrate × leaf area (variance = 0.00835) interactions were identified as important factors on rooting of *A. floribunda* single-node cuttings at week 38. Rooting medium, clone and leaf area had highly significant effects ($p < 0.001$). Application of auxin did not enhance rooting percentage in cuttings by the end of experiment. Optimising important factors (substrate, clone and leaf area) for rooting identified best rooting percentages (68.7%) in leafy cuttings from clone MB in sand. None of the tested factors had significant effects on number of roots, and rooted cuttings had on average one root. These results indicate that *A. floribunda* is difficult to root, but is amenable to vegetative propagation. Further work is required to achieve good rooting, as this study indicated that good rates of rooting can be achieved when important factors are optimised.

Keywords: Single-node leafy stem cutting; Auxin type; Clone; Leaf area; Non-mist propagator; Rooting media

1. Introduction

New initiatives in tropical forest tree improvement aim at developing cultivars of trees with desired fruit, nut and medicinal characteristics (Leakey et al., 2006). The "Cinderella species" overlooked by science (Leakey and Newton, 1994) now forming this worldwide program of indigenous tree domestication include *Allanblackia floribunda* Oliv. (Clusiaceae), with a special focus on Cameroon, Nigeria, Equatorial Guinea, Gabon and Democratic Republic of Congo (Leakey and Simons, 1998). These multi-purpose species have traditionally provided communities with their everyday needs for food and medicinal products (Leakey, 1999), and often have high market value regionally and internationally (Ndoye et al., 1998; Tabuna, 1999), as well as important social and livelihood benefits in local communities (Schreckenberg et al., 2002).

The bark of *A. floribunda* is locally used against coughs, dysentery, diarrhoea, toothache, and as an aphrodisiac and pain reliever. Consequently, the species is one of the most commonly used medicinal plants in Cameroon (Laird, 1996) and possibly has greater pharmaceutical potential. For example, Guttiferone F, an HIV-inhibitor, was found in extracts of *Allanblackia stuhlmannii* (Fuller et al., 2003). *A. floribunda* trees produce berry-like fruits with many seeds rich in a hard white fat (67.6–73% of seed mass, Foma and Abdala, 1985) consisting mostly of oleic and stearic acid (39–45 and 52–58%, respectively, Hilditch, 1958, quoted in http://www.fao.org/docrep/X5043E0d.htm). Oleic and stearic acids are reported to lower plasma cholesterol levels (Bonanome and Grundy, 1988), thus

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reducing the risks of heart attack. *Allanblackia* oil is also used in soap manufacture and is of commercial interest for margarine production, as it requires less chemical processing and refraction than palm oil. To investigate these opportunities, the World Conservation Union (IUCN), Netherlands Development Organisation (SNV), the World Agroforestry Centre (ICRAF) and Unilever plc have formed a partnership to produce nuts of *Allanblackia* species on a commercial scale. Currently, there have been no attempts to improve any of the species in this genus and there is only limited harvesting from wild stands.

Forest tree improvement programs have traditionally been timber-oriented and it is only in the last 10 years that high-value agroforestry trees, especially those for fruit/nut production, have been the subject of domestication and improvement of yield and quality (Leakey et al., 2006). Domestication utilises the variability within species, by selecting trees with desirable traits (Atangana et al., 2002) and propagating them sexually or asexually, depending on the appropriate strategy for the species and the products, while keeping a broad genetic base through gene banks. In the case of West and Central Africa, a clonal approach aimed at cultivar development has been used (Tchoundjeu et al., 2006). This has involved rooting leafy stem cuttings using inexpensive and low technology non-mist propagators (Leakey et al., 1990). These propagators consist of a wooden frame enclosed in clear polythene sheets so that the base of the propagator is watertight (3 m × 1 m × 1 m), and the lid is hermetically sealed. In parallel with this approach, studies aimed at identifying elite trees for propagation and cultivar development (Atangana et al., 2002; Leakey et al., 2005) have examined the tree-to-tree variability in a number of relevant traits aimed at the identification of market-oriented 'ideotypes' as a guide to the genetic selection (Leakey and Page, 2006). To domesticate *A. floribunda*, vegetative propagation methods need to be developed to allow the multiplication of selected genotypes, as the species is allogamous (Keay, 1989). Although considerable information is available on vegetative propagation of tropical African tree species using simple and inexpensive technologies (Leakey et al., 1990; Leakey, 2004), not much is known about vegetative propagation of *Allanblackia* species. The present paper investigates the effects of important factors affecting rooting ability of juvenile leafy stem cuttings of *A. floribunda* from coppicing stumps, using non-mist propagators. The factors investigated are (i) clone, (ii) rooting medium, (iii) auxin, (iv) leaf area, and their interactions.

2. Materials and methods

2.1. Study species

Mature *A. floribunda* is a medium-sized forest tree species of about 30 m in height and a diameter at breast height of about 80 cm, with a natural range from Cameroon to Democratic Republic of Congo (Vivien and Faure, 1996). Trees of the species are found in evergreen lowland and deciduous forests, and have many-seeded (40–100 seeds per fruit) berry-like, brown fruits up to 15–25 cm long, hanging on long pedicels (Vivien and Faure, 1996). The germination percentage of *A. floribunda* seeds has been reported to be less than 5% (Vivien and Faure, 1996).

Few studies on vegetative propagation of species in Clusiaceae are reported. Nyansi (2004) investigated the effects of rooting medium and leaf area on rooting of *Garcinia kola* leafy stem cuttings, whereas grafting in *Garcinia mangostana* is reported in http://www.montosogardens.com/mangosteen_grafting.htm.

2.2. Plant material samplings and cuttings preparation

The juvenile cuttings used in this experiment were harvested from four coppicing stumps from recently felled mature trees at Ngoumou (latitude: 03°61′N; longitude: 11°31′E; elevation: 695.6 m above sea level (asl)) in Cameroon, sprayed with water from a knapsack sprayer and stored in moist polythene bags for a maximum of 45 min before reaching the on-station nursery of ICRAF—African Humid Tropics Region in Nkolbisson, Yaoundé (latitude: 3°51′N, longitude: 11°25′E; altitude: 700 m asl). Yaoundé and Ngoumou are located in the semi-deciduous forest zone of low altitude in Cameroon (Letouzey, 1985). Average annual rainfall is 1692 mm and rainfall pattern is bimodal with two dry and two rainy seasons. Relative humidity varies generally between 73 and 84%, and average temperature is 25 °C. The stumps produced four to five young shoots 1 m in height. Using a sharp blade, pest and disease-free shoots were cut into single-node cuttings of 3–4 cm length. The number of cuttings taken per shoot varied depending on the number of nodes present.

2.3. Experimental design

Sixty-four cuttings from each stump were set in each of four replicate blocks arranged in a randomised split-split plot factorial design ($n = 1024$ cuttings: 64 cuttings × 4 clones × 4 replicates). Each main plot contained the four clones and was divided at random into two types of rooting medium (sand or a 50:50 mixture of sand/sawdust) such that there were 512 cuttings per rooting medium (8 cuttings × 4 shoots × 4 clones × 4 replicates). At the sub-plot level, four auxin treatments were tested: indole-3 acetic acid (IAA), indole-3-butyric acid (IBA), 1-naphtalene acetic acid (NAA) and alcohol (control), each cutting receiving 50 μg of auxins in 10 μl of alcohol, applied to the cutting base using a micropipette. Each auxin treatment was assigned at random to each sub-plot such that there were 256 cuttings per treatment (4 clones × 4 shoots × 2 cuttings × 2 media × 4 replicates). At the sub-sub plot level, four leaf areas were investigated: 0, 12.5, 25 and 50 cm^2. The leaf lamina of each cutting was trimmed with scissors using paper templates, and 8 cuttings assigned at random per sub-sub plot to give 256 cuttings per treatment (4 clones × 2 cuttings × 4 auxins × 2 media × 4 replicates).

Four high-humidity, non-mist poly-propagators (as described by Leakey et al., 1990) were used in this experiment. The propagation media were treated with fungicide (Ridomil Plus (metalaxyl-M and cupper) and Caocobre (cupper): 50 g each dissolved in 15 l of water) and insecticide (Cyperdim 220

Fig. 1. Single-nodes leafy stem cuttings of *A. floribunda* rooted under non-mist propagators in ICRAF nursery, Yaoundé, Cameroon.

EC 200 g/l dimethoate and 20 g/l cypermethrine: 40 ml dissolved in 1.5 l of water) 3 days prior to the beginning of the experiment. Caocobre was applied to the rooting medium when necessary during the experiment. To maintain a low saturation deficit whenever the propagator lid was opened for inspection, cuttings were sprayed with a fine jet of water. Assessments of rooting success were done once every 2 weeks after the first 10 weeks by lifting the cuttings from the rooting medium. A cutting was considered to be rooted (Fig. 1) when it had one or more roots exceeding 1 cm; the number of roots was also recorded. Rooted cuttings were removed from the experiment and potted in black polythene bags (1 l) containing a 2:1 mixture of forest soil and sand. Cuttings were defined as "dead" when severely rotted. The experiment was terminated after 38 weeks. Each cutting has either rooted or not, and coded 0 or 1.

2.4. Statistical analyses

Data was collated in MS Excel spreadsheets and subjected to REML variance components analyses using the linear mixed model dialogue in GenStat for Windows 6th edition software package for estimation of variance used to identify important factors affecting rooting ability (percentage of cuttings rooted, percentage of cuttings 'dead') in *A. floribunda* leafy stem cuttings. The fixed model was assumed constant and random model fitted as follows:

$$Y_{ijk\ln} = \mu_i + \pi_j + \beta_k + \lambda_l + \delta_n + (\pi\beta\lambda\delta)_{ijk\ln} + \varepsilon_{ijk\ln}$$

where μ is the effect of block i, π the effect of clone j, β the effect of substrate k, λ the effect of hormone l, δ the effect of leaf area n, $\pi\beta\lambda\delta$ the interaction of clone i, substrate k, hormone l and leaf area n, ε being the residual.

Statistical analyses were performed using the Generalised Linear Models (GLM) dialogue in GenStat for Windows 6th edition computer software package. Owing to the binomial distribution of rooting data, a logistic regression approach was used to perform analyses of deviance using the following model:

$$\text{logit}(p_{ijk\ln}) = \log\left(\frac{p_{ijk\ln}}{1 - p_{ijk\ln}}\right) = \alpha + \mu_i + \pi_j + \beta_k + \lambda_l + \delta_n + (\pi\beta\lambda\delta)_{ijk\ln} + \varepsilon_{ijk\ln}$$

where α is the overall mean, μ the effect of block i, π the effect of clone j, β the effect of substrate k, λ the effect of hormone l, δ the effect of leaf area n, $\pi\beta\lambda\delta$ the interaction of clone i, substrate k, hormone l and leaf area n, ε being the residual, and p the probability of a cutting rooting. Identified important effects from REML analyses were fitted in the model to predict response variate (proportion of cuttings rooted).

The number of roots per rooted cuttings had a Poisson distribution as it was a count, and analyses were performed fitting the following statistical model:

$$\log(\text{number of roots}) = \alpha + \mu_i + \pi_j + \beta_k + \lambda_l + \delta_n + (\pi\beta\lambda\delta)_{ijk\ln} + \varepsilon_{ijk\ln}$$

where α, μ, π, β, δ, $\pi\beta\lambda\delta$, and ε are effects previously described.

3. Results

3.1. Percentage of cuttings rooted

Clone × substrate, clone × substrate × leaf area and substrate × leaf area interactions were identified as important factors (variance = 0.01706, 0.00835 and 0.00427, respectively) influencing the rooting of *A. floribunda* leafy stem cuttings (Table 1) at week 38. Substrate, clone and leaf area individually, also influenced rooting in *A. floribunda* leafy stem cuttings at week 38 (Table 1).

Table 1
Factors affecting rooting percentage in *A. floribunda* leafy stem cuttings in non-mist propagators in the ICRAF nursery, Yaoundé, Cameroon, at 38 weeks

Random term	Estimated variance component
Replicate	0.00021
Clone	0.005473
Substrate	0.00814
Hormone	0.00000
Leaf area	0.00077
Clone × substrate	0.01706
Substrate × hormone	0.00000
Hormone × leaf area	0.00005
Clone × hormone	0.00000
Clone × leaf area	0.00255
Substrate × leaf area	0.00427
Clone × substrate × leaf area	0.00835
Substrate × hormone × leaf area	0.00000
Clone × substrate × hormone	0.00000
Clone × hormone × leaf area	0.00000
Clone × substrate × hormone × leaf area	0.00000
Residual	0.0553

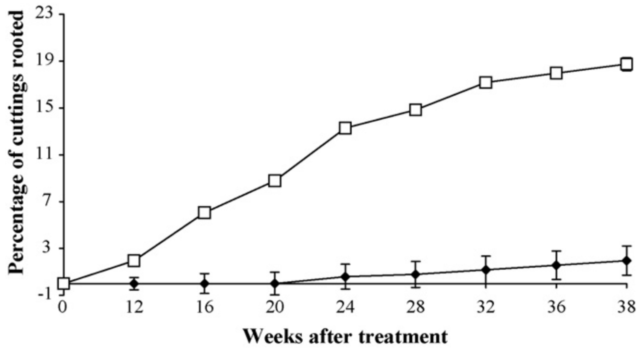

Fig. 2. Effects of different rooting media on rooting percentages of *A. floribunda* single-node leafy stem cuttings in ICRAF nursery, Yaounde, Cameroon. Error bars = ±S.E. (□ = sand and ◆ = 50:50 mixture of sand/sawdust).

Cuttings rooted better in sand (18.7 ± 1.25% at 38 weeks) than in the 50:50 mixture of sand/sawdust (1.9 ± 0.55% at 38 weeks, Fig. 2). The best rooting percentage was observed in cuttings from clone MB (28.5 ± 1.89%) and the worst in cuttings from clone NG1 (1.6 ± 0.73%). Clone also affected the start of rooting, as cuttings from clone MB started to root 10 weeks after the experiment was set up, while those of clones NG1 and NG4 occurred 10 weeks later (Fig. 3). Leafy cuttings (12.5, 25 and 50 cm^2) started to root 10–12 weeks after the experiment was set up, while leafless cuttings did not root throughout the experiment (Fig. 4). Leaf area in leafy cuttings did not affect rooting ability at 38 weeks (14.8 ± 1.69, 13.3 ± 1.57 and 13.3 ± 1.44% for 50, 25 and 12.5 cm^2, respectively).

Best rooting percentages were observed in leafy cuttings from clone MB in sand (68.7 ± 7.99, 68.7 ± 7.85 and 68.7 ± 7.21% for 50, 25 and 12.5 cm^2, respectively) at the end of the experiment. However, this rooting percentage was obtained with 25 cm^2 cuttings from clone MB in sand at week 24 (Fig. 5).

Substrate and clone had highly significant ($p < 0.001$) effects on rooting percentage of *A. floribunda* leafy stem cuttings from weeks 12 to 38. Leaf area had significant ($p = 0.050$) effects at week 12 and highly significant ($p < 0.001$) effects from weeks 16 to 38 on rooting percentage of *A. floribunda* cuttings. Auxins had significant ($p = 0.041$) effects on rooting percentage at week 12 and no significant ($p = 0.66$) effects from weeks 16 to 38 ($p = 0.889$). Interacting all tested factors had no effects ($p = 0.998$) on rooting percentage of *A. floribunda* leafy stem cuttings.

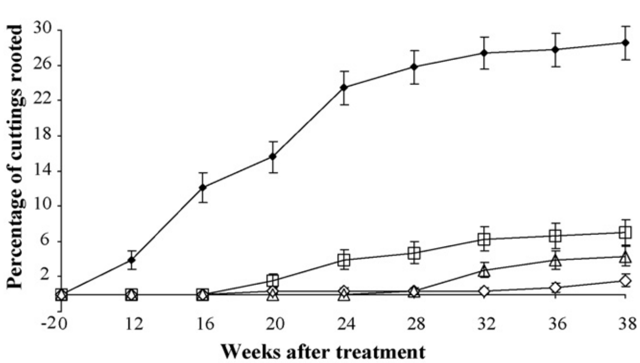

Fig. 3. Effects of different clones on rooting percentages of *A. floribunda* single-node leafy stem cuttings in ICRAF nursery, Yaounde, Cameroon. Error bars = ±S.E. (◆ = MB; ◇ = NG1; △ = NG2; □ = NG4).

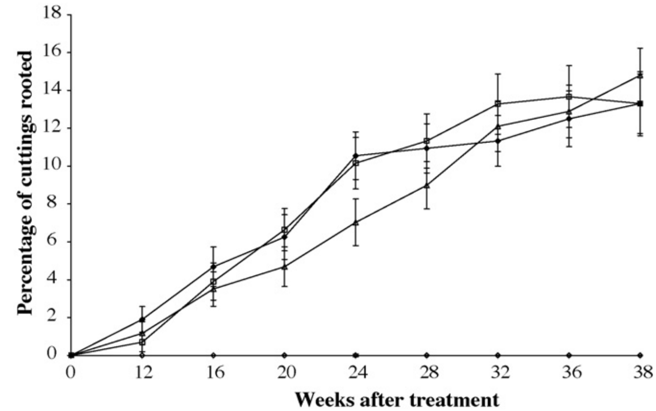

Fig. 4. Effects of different leaf areas on rooting percentages of *A. floribunda* single-node leafy cuttings in ICRAF nursery, Yaounde, Cameroon. Errors bars = ±S.E. (◇ = 0 cm^2; △ = 12.5 cm^2; ◆ = 25 cm^2; □ = 50 cm^2).

3.2. Number of roots

None of the factors tested had significant effects on the number of roots of rooted *A. floribunda* cuttings at week 38 ($p = 0.917$, 0.562, 0.448 and 0.927 for clone, substrate, leaf area and hormone, respectively), as rooted cuttings had on average one root. No rooted leafless cutting was recorded.

4. Discussion

The importance of rooting medium for rooting of cuttings is widely recognised (Leakey et al., 1990; Mesén et al., 1997b; Hartmann et al., 2002; Tchoundjeu et al., 2004). Evidence from this study indicates that rooting medium strongly affects rooting percentages of *A. floribunda* cuttings, sand being the best. This contrasts with the high rooting percentages observed in the water-retentive sawdust by Mialoundama et al. (2002) and Tchoundjeu

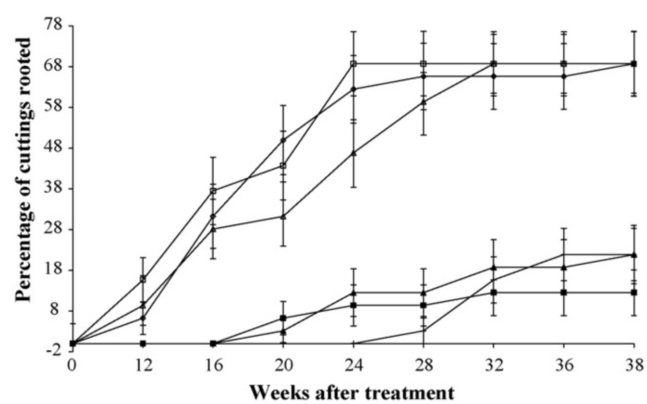

Fig. 5. Effects of sand, clones and leaf areas on rooting of single-node leafy stem cuttings of *A. floribunda* in non-mist propagators in ICRAF nursery, Yaounde, Cameroon. Errors bars = ±S.E. (△ = clone MB × sand × 12.5 cm^2; + = clone NG2 × sand × 12.5 cm^2; ■ = clone NG4 × sand × 12.5 cm^2; ◆ = clone NG4 × sand × 12.5 cm^2; □ = clone MB × sand × 25 cm^2; ◇ = clone MB × sand × 50 cm^2; ▲ = clone NG4 × sand × 50 cm^2).

et al. (2002, 2004) for *Dacryodes edulis*, *Prunus africana* and *Pausynistalia johimbe*, respectively. However, high rooting percentages were identified in sand media in *Cordia alliodora* (Leakey et al., 1990). Sand was also identified as best rooting medium for *G. kola* leafy stem cuttings (Nyansi, 2004). Between-species difference in rooting ability is explained by their xeromorphic or hydromorphic status (Loach, 1985), and the effects of this on the water relations of the cuttings (Mesén et al., 1997a). An appropriate rooting medium generally has an optimal volume of gas-filled porespace and an oxygen diffusion rate adequate for the needs of respiration (Andersen, 1986). As suggested for *Chrysanthemum* cuttings (Hartmann et al., 2002), the poor rooting percentage recorded for *A. floribunda* cuttings in 50:50 mixture of sand and sawdust may reflect anoxia associated with high water contents.

Genetic potential, as well as propagation environment, post-severance treatment, cutting origin and environment, stockplant physiology and management, have been reported to influence rooting (Hartmann et al., 2002; Leakey, 2004). Differences in rooting ability between clones could be due to genetic differences in cutting morphology and/or physiology (Leakey, 2004), or else be genetically controlled (Marques et al., 1999). Results from the present study indicate that rooting ability in *A. floribunda* is affected by clonal differences. Such differences in rooting ability have also been reported for some forest tree species (*Platanus occidentalis*, Cunningham, 1986; *Albizia guachapele*, Mesén et al., 2001). Hoad and Leakey (1994, 1996), in contrast, found that in *Eucalyptus grandis*, manipulating stockplant light environments in ways that changed the morphology and physiology of the cuttings enhances rooting in reputed "difficult-to-root clones". Also, genetic variation in rooting potential in *Triplochiton scleroxylon* was minimised when optimising pre-severance (nutrient application) and post-severance factors (length of the new shoot, total leaf area and within shoot position; Dick et al., 2004). Further work is required to clarify whether observed between-clones variation in rooting ability in *A. floribunda* can be minimised when interacting propagation environment and pre- and post-severance factors. *A. floribunda* cuttings mainly root between weeks 16 and 24, while most single-node leafy stem cuttings of a wide range of tropical trees can be successfully rooted within 6–8 weeks (Leakey et al., 1982), indicating that *A. floribunda* is seemed to be 'recalcitrant' plant (Hartmann et al., 2002).

Auxins are reported to play a significant role in stimulating root initiation in stem cuttings of woody plants (Tchoundjeu et al., 2002, 2004). Differences in rooting frequency depending on exogenous auxins or a combination of auxins used have been reported (Klass et al., 1987; Poupard et al., 1994). The lack of any significant improvement of rooting percentage by auxin application in *A. floribunda* is unusual for single-node cuttings of tropical trees, although application of NAA or a range of IBA concentrations showed no clear response on rooting percentage of *Lovoa trichiloides* cuttings (Tchoundjeu, 1989; Leakey et al., 1990). The reason why auxin application did not enhanced rooting of cuttings in *A. floribunda* cuttings is unknown. Further studies on the effects of a range of auxin concentrations on rooting of *A. floribunda* single-node leafy stem cuttings are suggested, as *L. trichilioides* cuttings rooted better at high auxin concentrations (Tchoundjeu, 1989).

Rooting of softwood cuttings usually depends on the presence of a leaf, as cuttings have to produce assimilates faster than they are losing them through respiration to root (Mesén et al., 1997a). As found in many other temperate species, the rooting ability of cuttings from tropical trees like *Khaya ivorensis* (Tchoundjeu and Leakey, 1996), *P. africana* (Tchoundjeu et al., 2002), *Irvingia gabonensis* (Shiembo et al., 1996a), *P. johimbe* (Tchoundjeu et al., 2004), *G. kola* (Nyansi, 2004), are strongly affected by leaf area. This was not so apparent in *A. floribunda* although leafy rooted much better than leafless cuttings. No optimum rooting leaf area was found in *A. floribunda* cuttings, what is unusual and contrasts with other tropical trees (25–50 cm^2 for *G. kola*, 50 cm^2 for *P. johimbe* and *T. scleroxylon*, 200 cm^2 for *Lovoa trichilioides*; Nyansi, 2004; Tchoundjeu et al., 2004; Leakey et al., 1982; Tchoundjeu and Leakey, 2001). Rooting in *A. floribunda* is possible only in leafy stem cuttings as opposed to leafless stem cuttings. A similar observation was reported for *K. ivorensis* with a lower optimal leaf area of 10 cm^2 (Tchoundjeu and Leakey, 1996).

Rooting of leafy cuttings seems to be affected by a combination of factors influencing root formation (Dick and Dewar, 1992). In *A. floribunda* increase in rooting percentage of leafy stem cuttings was observed by combining the factors of clone and rooting media. Interacting hormone and species, and hormone and provenance within species in *Racosperma auriculiforme* and *Racosperma mangium* also enhanced rooting percentage in cuttings (Khasa et al., 1995). Though being difficult to root, *A. floribunda* can be propagated using non-mist propagators, and further investigation is needed to reduce time to root and increase rooting percentage. Further work is also required to identify: optimal environmental conditions to achieve good rooting; optimum pre- and post-severance treatments; optimal morphological and physiological condition of the cutting material (as affected by stockplant environment, Dick et al., 2004; Leakey, 2004). This study has indicated that higher rooting percentages can be achieved when important factors are optimised.

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