

Preliminary survey of clonal variation in rooting of *Allanblackia floribunda* leafy stem cuttings

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Abstract: Clonal and cutting stem morphology variations in rooting ability were simultaneously examined in *Allanblackia floribunda* Oliv. using leafy stem cuttings in two experiments. The hypothesis tested is that clonal variation in rooting percentage is due to variation in cutting morphology. Each of the four replicate blocks used per experiment consisted of 18 treatments from two crossed factors, diameter or length \times clone, making a total of $n = 216$ cuttings (3 cutting lengths (3, 4, and 5 cm) or diameters (2, 3, and 4 mm) \times 6 stumps (clones) \times 12 cuttings, as each experimental unit consisted of 12 cuttings). The cuttings were used for rooting in nonmist propagators. No clone \times cutting diameter and clone \times cutting length interactions ($P = 0.293$ and 0.513 , respectively) on rooting percentage were found at the end of the study. Highly significant and significant clonal variations in rooting percentage were noted throughout the experiments. Results from this study reject the hypothesis being tested. Finally, further work should be done on the effects of physiological status of the cuttings and clonal variation in this species.

Résumé : La variabilité de l'aptitude à l'enracinement en lien avec le clone et la morphologie des boutures de tiges chez *Allanblackia floribunda* Oliv. a été étudiée simultanément au moyen de deux expériences avec des boutures de tiges feuillées. Les auteurs ont testé l'hypothèse voulant que la variation clonale du pourcentage d'enracinement soit due à la variation morphologique des boutures. Chaque expérience était constituée de quatre répétitions en blocs contenant chacun 18 traitements résultant de deux facteurs croisés, le diamètre ou la longueur des boutures par le clone, pour un total de $n = 216$ boutures (3 longueurs (3, 4 et 5 cm) ou diamètres (2, 3 et 4 mm) \times 6 cépées (clones) \times 12 boutures, de telle sorte que chaque parcelle était constituée de 12 boutures). Les boutures furent enracinées dans des chambres de croissance sans brumisation. Aucune interaction clone \times diamètre des boutures et clone \times longueur des boutures (respectivement, $P = 0,293$ et $0,513$) dans le pourcentage d'enracinement ne fut notée à la fin de l'étude. Des variations clonales du pourcentage d'enracinement significatives et fortement significatives furent observées lors des deux expériences. Les résultats de cette étude invalident l'hypothèse qui a été testée. Finalement, les auteurs concluent que des travaux supplémentaires portant sur les effets de l'état physiologique des boutures et de la variation clonale chez cette espèce devraient être entrepris.

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Introduction

Vegetative propagation, or cloning, is of practical value in tree improvement programs, as it offers greater genetic gain through broad-sense heritability and allows genetic evaluation of genotypes and their interaction with the environment through clonal testing (Foster and Shaw 1988; Zobel and Talbert 1991). The development of appropriate protocols for cloning requires investigation of factors playing an important role in the rooting process. Factors influencing the ability of leafy softwood stem cuttings to induce adventitious root formation (termed "rooting") were reviewed by Leakey (2004). These include the propagation environment, postseverance treatments (auxin application, leaf area, cutting length), stockplant factors (cutting origin and environment), preseverance stockplant environment, stockplant management, phase change (ontogenetic and physiological ageing), and genetic variation, which was attributed to between-tree genetic changes in morphology and physiology of cuttings (Hoad and Leakey 1994, 1996; Dick et al. 2004). Hoad and Leakey (1994, 1996) found that in *Eucalyptus grandis* W.

Hill ex Maiden, manipulating stockplant light environment affected dry matter partitioning and significantly modified the morphology and physiology of the cuttings, enhancing rooting in reputed "difficult-to-root" clones. Also, Dick et al. (2004), using stepwise regression analyses, observed that genetic variation in rooting potential in *Triplochiton scleroxylon* K. Schum was minimized by optimizing nutrient application and length of the new shoot, total leaf area, and node position. They concluded that genetic differences in rooting ability are mediated through genetic differences in cutting morphology or physiology within species.

Clonal variation in rooting traits of cuttings was observed in western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) cuttings (Foster et al. 1984), loblolly pine (*Pinus taeda* L.) (Foster 1990; Baltunis et al. 2005), *Pinus elliottii* Engelm var. *elliottii* Little and Dorman \times *Pinus caribaea* Morelet var. *hondurensis* Barrett and Golfari hybrid families (Shepherd et al. 2005), hybrid larch (*Larix decidua* Mill. \times *Larix kaempferi* (Lamb) Carr.) (Radosta et al. 1994), and *Eucalyptus globulus* Labill (Lemos et al. 1997). Khasa et al. (1995)

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observed an amplification of provenance variation by exogenous auxin in the rooting ability of *Racosperma auriculiforme* Cunn ex Benth. and *Racosperma mangium* (Willd.) Pedley. Clonal variation in rooting ability has been attributed to genetic and “C” effects, the latter defined as the physiological or morphological characteristics unique to the ortet and reflecting its growth environment (Lerner 1958; Foster et al. 1984; Radosta et al. 1994). Moderate narrow-sense heritability ($h^2 = 0.54$) was identified in *E. globulus* (Lemos et al. 1997), whereas Radosta et al. (1994) identified a genetic contribution of 36% for the percentage of rooting in hybrid larch. Rooting traits were found to be highly heritable in *T. heterophylla* cuttings ($H^2 = 0.87$ – 0.92) (Foster et al. 1984) and in *P. elliotii* × *P. caribaea* families ($H^2 = 0.62$ – 0.68) (Shepherd et al. 2005) and moderately heritable in *P. taeda* ($h^2 = 0.46$ and $H^2 = 0.40$, respectively) (Foster 1990). In contrast, individual-tree heritabilities of rooting percentage were observed to be weak in five genomic groups of *Populus* ($h^2 = 0.09$ – 0.11) (Zalesny et al. 2005), indicating that the magnitude of genetic control of rooting traits also varies between species. Haissig et al. (1992), when reviewing the controls of adventitious rooting, postulated that these controls may be identified by searching for and studying direct genetic effects using current molecular technologies and new plant material. This paper reports an investigation on the simultaneous effects of clone and cutting stem morphology on the rooting of juvenile single-node leafy stem cuttings from coppicing stumps of *Allanblackia floribunda* Oliv. (Clusiaceae), a tropical tree valued for its seeds, which are used by the cosmetic and food industries. A nonmist system and inexpensive propagators (Leakey et al. 1990) were used because they did not require access to running water and electricity. Such simple and low-cost approaches are best suited for developing rural nurseries propagating high-value trees indigenous to the humid tropical regions of Africa (Tchoundjeu et al. 2006). This study is part of a wide program started in Cameroon in 2005 and aimed at improving species of the genus *Allanblackia*. Clonal approaches are used instead of breeding because of poor seed germination in the genus (germination rate is less than 5%; Vivien and Faure 1996). The present study builds on results from Atangana et al. (2006) indicating that *A. floribunda* is difficult to root and suggesting that further work must be carried out to clarify whether observed between-clone variation in the rooting ability in the studied species can be minimized when interacting propagation environment and pre- and postseverance factors, such as cutting size. The hypothesis tested in this study is that clonal variation in the rooting ability in *A. floribunda* single-node leafy stem cuttings is due to variation in cutting stem morphology.

Materials and methods

Two experiments were carried out in the course of this study to test the formulated hypothesis on the effects of cutting diameter and clone on rooting of leafy stem cuttings and effects of cutting length and clone on rooting of leafy stem cuttings.

Study species

Mature *A. floribunda* is a medium-sized forest tree species

of about 30 m in height with a diameter at breast height of about 80 cm that has a natural range from Cameroon to Democratic Republic of Congo (Vivien and Faure 1996). Trees of the species are usually found in evergreen lowland and deciduous forests and have berry-like brown fruits that are up to 15–25 cm long with many seeds (40–100 seeds per fruit) hanging on long pedicels (Vivien and Faure 1996). These seeds are rich in a hard white fat (67.6%–73% of seed mass; Foma and Abdala 1985) consisting mostly of oleic and stearic acids, which is a primary reason why they are purchased from local communities and used in the food and cosmetic industries.

Plant material samplings, cuttings preparation, and experiment setting

Cutting stem mainly consists of cutting length and diameter, ranked among the top postseverance factors influencing rooting ability (reviewed by Leakey 2004; Hoad and Leakey 1996; Tchoundjeu and Leakey 1996). Cutting length depends on internode length within the shoot, which decreases sequentially down a stem in some species. From the top to the bottom of the shoots, rooting ability of a cutting decreases, while mortality increases, as found in *T. scleroxylon* (Leakey and Mohammed 1985).

Plant material sampling and cuttings preparation were done similarly to as described in Atangana et al. (2006). The juvenile material used in this study was harvested from shoots on top of each of 12 coppicing stumps (Tchoundjeu 1989) with appropriate characteristics for the experiments to be carried out on recently felled natural and unselected mature trees at the Ngoumou site in Cameroon (3°61'N, 11°31'E; elevation 695.6 m above sea level). The cuttings were 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 the World Agroforestry Centre (ICRAF), West and Central Africa Region/African Humid Tropics in Nkolbisson, Yaoundé (3°51'N, 11°25'E; elevation 700 m above sea level). Yaoundé and Ngoumou are located in the semideciduous forest zone of low altitude in Cameroon (Letouzey 1985). Average annual rainfall is 1962 mm, and the rainfall pattern is bimodal with two dry and two rainy seasons. Relative humidity varies generally between 73% and 84%, and the average temperature is 25 °C. The stumps produced four or five young shoots 1 m in height. Using a sharp blade, pest- and disease-free shoots were cut into single-node cuttings, the length varying according to the treatment applied. At most, three cuttings were taken per shoot from the second internode to the fourth one, as Leakey and Mohammed (1985) found that optimal rooting for *T. scleroxylon* was obtained in cuttings harvested within the second to the fourth internode down a stem. Cutting length and width were measured using calipers graduated to 0.1 mm. Leaf area of each cutting was trimmed to 25 cm², as it was found in a previous study that leafy cuttings root better than leafless ones (Atangana et al. 2006).

In the nursery, cuttings were inserted for rooting in nonmist 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. Sand was used as rooting medium following results from a previous study (Atangana et al. 2006).

Table 1. Type III tests of fixed effects and contrasts of rooting percentage of *Allanblackia floribunda* leafy stem cuttings in a factorial diameter × clone experiment at different weeks after inserting cuttings in nonmist propagators.

Source of variation	df	Week 22*		Week 26*		Week 30	
		F	P	F	P	F	P
Diameter	2	1.90	0.229	0.95	0.437	0.34	0.723
Clone	5	12.67	<0.001	12.57	<0.001	19.65	<0.001
Diameter × clone	10	0.88	0.563	1.12	0.377	1.26	0.293
Diameter linear	1	0.52	0.499	1.63	0.249	0.60	0.469
Diameter quadratic	1	3.29	0.120	0.27	0.619	0.09	0.776

*On rank-transformed data.

Experimental design

Six coppicing stumps distanced at least 200 m from each other were used for each experiment, each constituting a genetic origin. Thirty-six cuttings from each stump were set in each of four replicate blocks arranged in a factorial treatment structure. In each experiment, the two factors were then crossed, thus appearing to constitute a 3 × 6 factorial (3 cutting lengths (3, 4, and 5 cm) or diameters (2, 3, and 4 mm) × 6 stumps (clones) × 4 replicates × 12 cuttings, as each experimental unit consisted of 12 cuttings) experiment with 18 treatments per block. When investigating the effects of clone and cutting length on the rooting of cuttings, cutting diameter was fixed at 2 mm and clones C1, C2, C3, C4, C5, and C6 were used, while in the experiment on the effects of clone and cutting diameter, cutting length was fixed at 5 cm and clones C7, C8, C9, C10, C11, and C12 were used. Nonmist polypropagators (Leakey et al. 1990) were used as propagation environment. Assessments of rooting success started from the first 10 weeks, as *A. floribunda* is a difficult-to-root species (Atangana et al. 2006), and was done once every 4 weeks. A cutting was considered to be rooted when it had one or more roots exceeding 1 cm. Rooted cuttings were coded 1 and nonrooted ones 0. The same code was used for “alive” and “dead” cuttings. A cutting was considered “dead” when severely rotted. The number of roots was not used for analysis, as rooted *A. floribunda* cuttings have an average of one root (Atangana et al. 2006).

Statistical analyses

Data consisting of rooting percentage per experimental unit was submitted to ANOVA using the Statistical Analysis System (SAS Institute Inc. 2006). Treatment effects were considered fixed and block effects assumed random, making the model mixed in each experiment. Prior to statistical analysis, the homogeneity of residual variances was tested graphically using proc plot in SAS. The normality of experimental errors was tested using proc univariate in SAS, and proc rank was applied where necessary (Conover and Iman 1981). For both experiments, the ANOVA was performed using the mixed-model procedure in SAS (Littell et al. 1996) following the statistical three-factor mixed model

$$Y_{ijk} = \mu + \pi_i + \alpha_j + \beta_k + (\alpha\beta)_{jk} + (\pi\alpha)_{ij} + (\pi\beta)_{ik} + (\pi\alpha\beta)_{ijk} + e_{ijk}$$

where Y_{ijk} is the average value of the dependant variable for the i th block, j th cutting diameter or length, and k th clone, μ is the overall mean, α_j , β_k , and $(\alpha\beta)_{jk}$ collectively represent

fixed effects and correspond, respectively, to cutting diameter or length, clone, clone cutting diameter or length × clone interaction, and π_i , $(\pi\alpha)_{ij}$, $(\pi\beta)_{ik}$, $(\pi\alpha\beta)_{ijk}$, and e_{ijk} collectively represent random effects and correspond, respectively, to block, block × cutting diameter or length interaction, block × clone interaction, block × cutting diameter or length × clone interaction, and the accepted random error. This model was further restricted, the block × diameter × clone interaction effect dropped from the full model, as the sum of the interaction effects over the levels of the fixed factor equals zero (Montgomery 2001). As diameter and length were quantitative variables, linear and quadratic contrasts were constructed for a priori comparison of levels of treatments. Pairwise comparison of treatment means in other variables studied were obtained using the pdiff option on the least square means statement of the mixed procedure in SAS. From week 18 downwards, experimental errors were not normally distributed in the two experiments after rank transformation of data; for this reason, these data were not submitted to ANOVA.

Results

Effects of cutting diameter and clone on rooting percentage

This experiment lasted for 30 weeks. Convergence criteria were met for each data set, and Type III tests of fixed effects are given in Table 1. No diameter × clone interaction ($P = 0.563$, 0.377 , and 0.293 at weeks 22, 26, and 30, respectively) was noted on rooting percentage throughout the experiment. Clone had highly significant ($P < 0.001$) effects on proportion of cuttings rooted from week 22 to week 30. In contrast, cutting diameter did not ($P = 0.229$, 0.437 , and 0.723 at weeks 22, 26, and 30, respectively) influence rooting percentage in *A. floribunda* leafy stem cuttings. Also, polynomial contrasts in cutting diameter did not identify (Table 1) significant differences in the percentage of cuttings rooted. Pairwise comparison of clones identified best rooting percentages in cuttings from C6 (Table 2), as they have a rooting ability different from that of other clones tested in this experiment. Linear and quadratic components of cutting diameter did not significantly influence rooting percentage in *A. floribunda* leafy stem cuttings at weeks 22 ($P = 0.449$ and 0.120 , respectively), 26 ($P = 0.249$ and 0.619 , respectively), and 30 ($P = 0.469$ and 0.776 , respectively).

Effects of cutting length and clone on rooting percentage

This experiment lasted for 26 weeks. Convergence criteria

Table 2. Effects of clone on rooting percentage of *Allanblackia floribunda* leafy stem cuttings 30 weeks after inserting cuttings in nonmist propagators in an experiment investigating the effects of cutting diameter \times clone on rooting of leafy stem cuttings (estimate, least square mean).

Treatment	df	Estimate \pm SE	<i>t</i>	Pr > <i>t</i>
C1	15	6.25 \pm 4.090	1.53	0.147
C2	15	4.86 \pm 4.090	1.19	0.253
C3	15	2.78 \pm 4.090	0.68	0.507
C4	15	0.69 \pm 4.090	0.17	0.867
C5	15	7.64 \pm 4.090	1.87	0.081
C6	15	47.9 \pm 4.090	5.31	0.002

were met for each data set, and Type III tests of fixed effects are given in Table 3. Length \times clone interaction did not significantly ($p = 0.43$ and 0.513 at weeks 26 and 22, respectively) influence rooting percentages of *A. floribunda* leafy stem cuttings throughout the experiment. Clone had a highly significant ($P = 0.008$) and significant ($P = 0.019$) effects on rooting percentage at weeks 26 and 22, respectively. In contrast, cutting length did not ($P = 0.789$ and 0.803 at weeks 26 and 22, respectively) influence rooting percentage in *A. floribunda* leafy stem cuttings. Pairwise comparison of clones identified best rooting percentages in cuttings from C11 (Table 4). Linear and quadratic components of cutting length had no effects on rooting percentage of *A. floribunda* leafy stem cuttings at weeks 22 ($P = 0.676$ and 0.628 , respectively) and 26 ($P = 0.888$ and 0.518 , respectively).

Discussion

The rooting of leafy stem cuttings involves a complex interaction of many factors. Factorial designs have the advantage of assessing the relative importance of factors, and they constitute an independent test for the factors being studied (Steel et al. 1997). This paper reports the first fine-scale study on the simultaneous effects of cutting stem morphology and clone on rooting of *A. floribunda* leafy stem cuttings. No cutting stem morphology \times clone interaction was identified in this study, indicating that there is no differential effect of one factor over the other. Genetic origin and cutting stem morphology effects on the proportion of *A. floribunda* rooted cuttings were not found to be related, indicating that clonal variation in cuttings, if found, is not minimized by cutting stem morphology.

Leakey (2004), drawing heavily on studies from *T. scleroxylon*, listed cutting length/diameter as one of the most postseverance factors affecting rooting of leafy stem cuttings. Cutting length, as well as cutting diameter, is associated with stored carbohydrates (Leakey et al. 1994), which influence the rooting potential of cuttings (Leakey and Mohammed 1985; Hoad and Leakey 1996; Mesén et al. 2001; Dick and Leakey 2006). The lack of effect of cutting stem morphology on the rooting of *A. floribunda* is unusual and unexplained and contrasts with the results of Tchoundjeu and Leakey (1996) in *Khaya ivorensis* A. Chev., Hoad and Leakey (1996) in *E. grandis*, and Dick et al. (2004) in *T. scleroxylon*. Further studies on the effects of cutting volume on rooting to better understand the effect of cutting stem morphology on the rooting ability in *A. floribunda* are

Table 3. Type III tests of fixed effects and contrasts of rooting percentage of *Allanblackia floribunda* leafy stem cuttings in a factorial cutting length \times clone experiment at different weeks after inserting cuttings in nonmist propagators.

Source of variation	df	Week 22*		Week 26	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Length	2	0.23	0.803	0.25	0.789
Clone	5	3.86	0.019	4.81	0.008
Length \times clone	10	0.94	0.513	1.05	0.430
Length linear	1	0.19	0.676	0.02	0.888
Length quadratic	1	0.26	0.628	0.47	0.518

*On rank-transformed data.

Table 4. Effects of clone on rooting percentage of *Allanblackia floribunda* leafy stem cuttings 22 weeks after inserting cuttings in nonmist propagators in an experiment investigating the effects of cutting length \times clone on rooting of leafy stem cuttings (estimate, least square mean).

Treatment	df	Estimate \pm SE	<i>t</i>	Pr > <i>t</i>
C7	15	0.695 \pm 1.927	0.36	0.724
C8	15	0 \pm 1.927	0	1
C9	15	6.36 \pm 1.836	3.46	0.003
C10	15	5.53 \pm 2.11	2.62	0.019
C11	15	11.11 \pm 1.927	5.77	<0.0001
C12	15	2.08 \pm 1.927	1.08	0.297

quired, as Hoad and Leakey (1996) reported influence of volume on rooting success.

Clonal variation in rooting percentage found in this study corroborates the results of Atangana et al. (2006), Mesén et al. (2001), Foster et al. (1984), Baltunis et al. (2005), Farmer et al. (1989), and Shepherd et al. (2005) in *A. floribunda*, *Albizia guachapele* (Kunth) Dug., *T. heterophylla*, *P. taeda*, balsam poplar (*Populus balsamifera* L.), and *P. elliotii* \times *P. caribaea* hybrid families, respectively. Furthermore, few genotypes (C6 and C11 in the first and second experiments, respectively) seem to have the potential for vegetative propagation from this study, although the number of clones tested in this study was low compared with 2200 and 596 clones for *P. taeda* studied by Baltunis et al. (2005) and Foster (1990), respectively. As found in *T. heterophylla* (Foster et al. 1984) and *L. decidua* \times *L. kaempferi* (Radosta et al. 1994), clonal variation for rooting traits in tree species could be due to genetic and nongenetic effects, the latter being the physiological or morphological characteristics unique to the ortet. Results from this study indicate that cutting stem morphological characteristics of the ortet do not amplify clonal variation in *A. floribunda*. Genetic variation of the root growth within a population had significant effects on fitness of individuals, and difference in fitness may be attributed to exploitation of slightly different soil layers (Ennos 1985). However, quantification and type of heritability in rooting of *A. floribunda* are required as genetic gain prediction is important in improvement of the species (Zobel and Talbert 1991).

The hypothesis tested would have been accepted whether there were highly significant effects of stem morphology and little or no effects of clone. Results from this study reject the hypothesis being tested and indicate that further

work is required to investigate the clonal variation in *A. floribunda* leafy stem cuttings by manipulating physiological status of cuttings and clone. Further studies combining pre- and post-severance factors to better understand the processes affecting rooting in this species and achieve good rooting are also recommended.

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