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Effective propagation of *Diospyros crassiflora* (Hiern) using twig cuttings

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Abstract

The African ebony (*Diospyros crassiflora*) is a well-sought commercial timber tree. As a result of intense harvesting pressure it ranks high on the IUCN' 1994 CITES list as endangered. Insufficient availability of seeds has limited the current efforts to propagate *D. crassiflora* from seedlings. Therefore, vegetative propagation through leafy stem cutting could be an alternative. The purpose of this study was to assess the effect of four rooting media, length of cuttings and auxin (seradix-2) application on the rooting ability of stem cuttings set up in a non-mist propagator. The rooting process was monitored over 14 weeks. Root development started after 9 weeks. At 14 weeks, the Seradix-2 and rooting medium significantly improved the rooting ability. Decomposed sawdust was the best substrate. The combination of the three factors didn't significantly influence rooting of cuttings. Stem cuttings of 5 cm length survive better in non-mist propagator. The results of the study suggest that *D. crassiflora* is amenable to vegetative propagation by leafy stem cutting.

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Introduction

The estimated forest area for Africa was 635 million hectares in 2005, representing 16 % of the global forest cover. Of this, the net annual forest loss in Africa between the years 2000-2005 has been estimated at 4 million hectares; nearly 55 % of the global deforestation (FAO, 2007). One of the most exploited timber in Cameroon is *Diospyros crassiflora*, commercially known as African Ebony. It is a hard black wood timber tree species belonging to the *Ebenenacea* family. Locally, the species is called *Kanran* in Nigeria or *Mevini* in Ewondo (Cameroon) (Vivien and Faure, 1994). The importance of the species in agroforestry has not yet been demonstrated, but it is a highly exploited species for diverse uses in household furniture and musical instruments (since 300 years). For instance, the African Ebony is used for accessories of violin, comps and glasses. It is also largely used for diverse joinery (Verbelen, 1999).

Due to considerable demand for *D. Crassiflora* in Europe, where 70 tons were respectively exported annually from Cameroon in 1960s (WCMC, 1991) and 35 m^3 Gabon in 1994, the non-sustainable exploitation of the resource has warranted the *International Union for Conservation of Nature (*IUCN) in 1994 to classify the species in the *Convention on International Trade in Endangered* (CITES) list, consequently forcing the governments of Cameroon and other producer countries to classify *D. crassiflora* among species requiring special authorization before exploitation (Verbelen, 1999). This policy has boosted governments' conservation strategy for the species in producer countries. In Cameroon for example, state, community and communal forests have been created and key stakeholders have to establish tree nurseries to regenerate the species through participatory tree domestication.

Domestication of agroforestry trees encompasses the socio-economic and biophysical processes involved in the identification and characterisation of germplasm resources; the capture, selection and management of genetic resources; and the regeneration and sustainable cultivation of the species in managed ecosystems (Simons and Leakey, 2004). This approach has been widely tested and used in sustainable forest resource management, especially for indigenous fruit trees (Tchoundjeu *et al.* (2008). The approach includes priority setting, germplasm collection, propagation and conservation (Akinnifesi *et al.*, 2008; Leakey and Akinnifesi, 2008; Tchoundjeu *et al.*, 2006). Many people propagate the species by seeds, but seed production is limited some years. As an alternative, vegetative propagation by leafy stem cuttings, air layering, grafting and tissue culture has been recommended. Based on comparative advantages, the most recommended technique is by leafy stem cuttings. The use of leafy stem cuttings has the advantage of yielding more plants from a single stock plant. For example, one plant from well managed *Khaya ivorensis* stock plant can provide about 10 new plants per year through leafy stem cutting techniques (Tchoundjeu, 1989). In addition, propagation by leafy stem cutting of young material generally produces a tree with a straight bole. Compared to tissue culture, propagation by leafy stem cuttings is cheaper and a simpler technique. This technique involves using seedlings or hedging a tree to generate stock plants from which young shoots are collected for cutting production. Stem cuttings are then placed in non-mist, high humidity propagators containing different substrates for rooting (Leakey *et al.,* 1990).
Previous research with a range of tropical tree species has identified a wide range of factors that influence adventitious root development in leafy stem cuttings. These include genotype, rooting medium, type and concentration of auxins, length of cutting, leaf area, shoot and node position for *Khaya ivorensis* and *Lovoa trichilioides* (Tchoundjeu, 1989)*, Dacryodes edulis* (Mialoundama *et al.,* 2002), *Irvingia gabonensis* (Schiembo et *al.,* 1996), *Ricinodendron heudelotii* (Schiembo *et al.,* 1997), *Pausinystallia johimbe* (Ngo Mpeck *et al.,* 2003), *Allanblackia floribunda* (Atangana *et al.,* 2006) and/or *Nauclea diderrichii* (Caspa *et al.,* 2009). In addition, many of these species display contrasting

responses to post-severance treatments. The purpose of this study was to access factors affecting the vegetative propagation of leafy stem cutting of *D. crassiflora*. Specifically, we assessed the effect of rooting medium, length of cuttings and the root-enhancing auxin (Seradix-2) on the rooting ability of *D. crassiflora* stem cuttings.

Materials and methods

Site, plant material and treatment

This study was conducted from April 2009 to January 2010 at ICRAF research nursery in Nkolbisson (3°52'N, 11°26'E), near Yaoundé, Cameroon. The climate is hot and humid, with an average temperature of about 25° C and a bi-modal rainfall regime ranging from 1,500 to 2,000 mm of rainfall per year. The experiment was carried out in a non-mist propagator developed by Leakey *et al.* (1990), installed in a shade house which had a mixture of translucent and corrugated iron roofing sheets and bordered with shade cloth allowing 70 % of the irradiance to enter the propagator.

Leafy stem cuttings of *D. crassiflora* were collected from 6-month old seedlings produced in the nursery. Before collecting the cuttings, the young plants were sprayed with water early in the morning. All cutting tools (pruners, knives) were disinfected before use. The juvenile parts were cut and conserved in a disinfected humid bag. In the nursery, leaves were cut to 50 cm^2 surface area. Stems were cut to either 2 or 5 cm in length, and had a circular base to guarantee the homogenous distribution of rooting and a slant-wise upper part to facilitate the runoff of water during spraying (Tchoundjeu, 1989). Seradix-2, a rooting hormone of IBA family was applied at the base of each treated cutting (except control). The treated cuttings were at once set randomly on three different substrates that had previously been disinfected with insecticide and fungicide: decomposed sawdust, river sand and mixture of 50% sand and 50% sawdust. In order to keep the cuttings cool, a light spray of water was applied on the foliage using a knap sack sprayer whenever the propagator was opened.

Experimental design

To achieve the objective of the study, a 3 x 2 x 2 factorial treatment structure was used in the trial. The 12 experimental treatments (Table I) consisted of combinations of all the levels of the three factors, namely: rooting media (decomposed sawdust, river sand, mixture of sand and sawdust) cutting length of 2 cm and 5 cm, and auxin treatment and control (untreated).

Due to the hierarchical nature of the experimental treatment and the layout of the propagator the experiment was laid out as a randomized complete block in split plot arrangement with three replicates. Each replicate was split into three main plots, and the three rooting media were randomly assigned to the main plots. Each of the three main plots was split into two subplots, and the two cutting length treatments were randomly assigned to the subplots. Finally, the subplots were split into two sub-subplots and the two hormone treatments were randomly assigned to the sub-subplots. Twelve cuttings were set in each sub-subplot, giving a total of 36 stem cuttings per treatment across the three replications and an overall total of 432 stem cuttings for the whole trial. The media were sprayed with fungicide with an active ingredient of demethoate, (50g/16 liter of water) and insecticide with cypermethrine as active ingredient (50 ml/16 liters of water) three days before the setting of cuttings.

Experiment monitoring and data collection

Every morning before 10.00 am, the propagator was opened and fallen leaves were removed. The water level was checked and adjusted if necessary, while the transparent plastic was cleaned and the cuttings sprayed with water. After one month, weekly assessments of rooting were carried out. Data collection ended after 14 weeks. The cutting was said to have rooted when it had one or more roots exceeding 1cm length. Rooted cuttings were removed from the experiment; their roots were counted before putting each of them into a polyethylene bag containing a 2:1 mixture of agricultural-field top soil and sand.

The response of each stem cutting was coded [rooted (1) or not (0), dead (1) or not (0)] from 4 weeks after setting to the 14th week. Dead cuttings were removed from the propagator while live ones were re-set for further observation the following week.

Statistical analysis

At the end of the trial, the total number of rooted cuttings, dead cuttings and roots formed were estimated for each treatment on weekly basis. The rate of rooting in each treatment was assessed using graphically tools of Excel 2007.

To determine the effect of experimental factors on the rooting ability of cuttings, the death rate and the number of roots developed per cutting, data collected were subjected to an analysis of deviance using Generalized Linear Models procedures of Genstat v.12 software. After the data was fitted into models, deviance was used to test the importance of the factors.

$$logit(p_{ijk}) = log\left(p_{ijk} / (1-p_{ijk}) \right) = Constant + S.effect + L.effect + H.effect + SL.effect + SH.effect + LH.effect + SLH.effect,$$

p_{ijk} is the rooting probability (yes or no) of cuttings in the substrate, the lenght treated with the the level of auxin.

S represents substrate, L the cutting lenght, H the auxin, SL, SH, LH and SLH the 2 and 3way interaction among factors.

For the death rate, same model is fitted but with p_{ijk} representing the probability of dying.

The same is valid with the number of roots except that the natural log function is used as a link fuction

The influence of rooting medium, cutting length, auxin and their interactions on rooting and mortality rates were assessed using the logistic regression model (equation 1) while a log linear regression model was fitted to the root number data assumed to follow *Poisson* distribution. Regression coefficients obtained were used to predict the average percentages of rooted and death cuttings per treatment combination. Factors having a significant effect on cutting rooting ability or mortality were compared among treatment levels using the Least Significant Difference (LSD) procedure.

Results

Rooting ability of D. crassiflora cutting

An analysis of deviance after fitting the model presented above (table 2) showed that two of the studied factors significantly affect the rooting ability of the *D. crassiflora* cuttings in the current study. Rooting medium had a consistent effect on the proportion of stems cuttings rooting over time $p<0.001$, $p=0.002$ and $p=0.003$ respectively at the 9th, 10th and 11th weeks after stem cutting were inserted in the non mist propagator. Auxin application also had an effect though only at the 11th week after stem cutting insertion *($p=0.02$)*. Stem cutting lengths as well as either 2 or 3-way interactions among the factors were not significant under the experimental conditions studied.

Table 1. Combinations of studied factors used as experimental treatments in the trial.

Substrate	Cutting length (cm)	Seradix-2 treatment	Experimental treatments
River sand only	2	Treated	RS2H1
		Not treated	RS2H0
	5	Treated	RS5H1
		Not treated	RS5H0
Decomposed sawdust only	2	Treated	SD2H1
		Not treated	SD2H0
	5	Treated	SD5H1
		Not treated	SD5H0
Mixture of river sand and sawdust	2	Treated	MI2H1
		Not treated	MI2H0
	5	Treated	MI5H1
		Not treated	MI5H0

Table 2. Accumulated analysis of deviance after fitting logistic regression model to the rooting probability of cuttings of *D. crassiflora*.

Variation source	Week 9	Week 10	Week 11
Block	0.001	<.001	0.18
rooting medium	<.001	0.002	0.003
Length	0.364	0.641	0.478
Auxin	0.088	0.331	0.024
Rooting medium*length	0.499	0.570	0.774
Rooting medium*auxin	0.240	0.457	0.644
Length*auxin	0.807	0.935	0.161
Rooting medium*length*auxin	0.478	0.483	0.893

Table 3. Cumulative rooting rate of 2 and 5 cm long *D. crassiflora* cuttings in three rooting medium at 9, 10 and 11 weeks after cuttings were planted.

Rooting substrates	Cutting length	9th week Seradix-2 treated cuttings	9th week Untreated cuttings	9th week Mean(se)	10th week Seradix-2 treated cuttings	10th week Untreated cuttings	10th week Mean (se)	11th week Seradix-2 treated cuttings	11th week Untreated cuttings	11th week Mean (se)
Decomposed Sawdust only	2 cm	25.29	12.70	17.97 a (4.12)	29.28	12.61	22.55a	50.23	37.72	43.13a (5.36)
	5 cm	16.94	16.94		24.08	24.08	(4.55)	60.87	23.69	
Mixture of River sand and sawdust	2 cm	25.29	25.29	20.04 a (3.92)	25.12	33.42	28.24a	33.55	33.55	37.72a
	5 cm	21.11	8.48		29.28	25.12	(4.36)	50.23	33.55	(4.87)
River sand only	2 cm	4.24	0.00	03.18 b (1.71)	16.79	8.41	09.46b	20.99	16.80	20.99b
	5 cm	8.48	0.00		8.41	4.21	(2.94)	29.37	16.80	(4.11)
Mean (se)		16.72 (2.99)	10.46 (2.43)		21.98 (3.36)	17.98 (3.08)		39.66 a (4.02)	26.92 b (3.71)	

Cell percentage are computed over a total of 36 cuttings;
In the margin column, percentages with the same letter are not significantly different at P= 0.05; s.e. standard error

The predicted percentages of rooted stem cuttings estimated from the model for each treatment combination and their marginal totals including their respective standard errors (s.e) for significant factors are presented in Table 3. Rooting media (50% river sand + 50% decomposed sawdust), register higher rooting rate of *D. crassiflora* leaf stem cutting; 20 and 18 % (week 9), 28 and 22 % (week 10) and 37 and 43 % (week 11) though not significant between both treatment. However, the total percentages of rooted cuttings were significantly lower (*p=0.05*) on river sand: 3, 10 and 21 % respectively at weeks 9, 10 and 11.

Table 4. Accumulated analysis of deviance after fitting logistic model to mortality rate data taking experimental factors and interactions as independent variables.

Variation source	d.f.	Deviance	Mean deviance	Deviance ratio	Approximate chi-2 probability
Block	2	0.238	0.119	0.12	0.888
Rooting medium	2	0.274	0.137	0.14	0.872
Length	1	2.825	2.825	2.83	0.063*
Auxin	1	1.438	1.438	1.44	0.230
Rooting medium * Length	2	2.613	1.307	1.31	0.271
Rooting medium * Auxin	2	2.080	1.040	1.04	0.353
Length * Auxin	1	0.309	0.309	0.31	0.578
Rooting medium * Length * Auxin	2	6.726	3.363	3.36	0.035*
Residual	20	12.579	0.629		
Total	33	29.083	0.881		

With respect to the effects of auxins, *D. crassiflora* stem cuttings treated with Seradix-2 had a higher rooting ability than untreated stem cuttings: 17, 30 and 40 % against 10, 18 and 28 % respectively at week 9, 10 and 11. The difference between treated and untreated stem cuttings was significant (p = 0.024) but only at the 11th week. For treated stem cuttings and irrespective of rooted medium, the 5 cm long stem cutting rooted better than the 2 cm long cutting (Table 3).

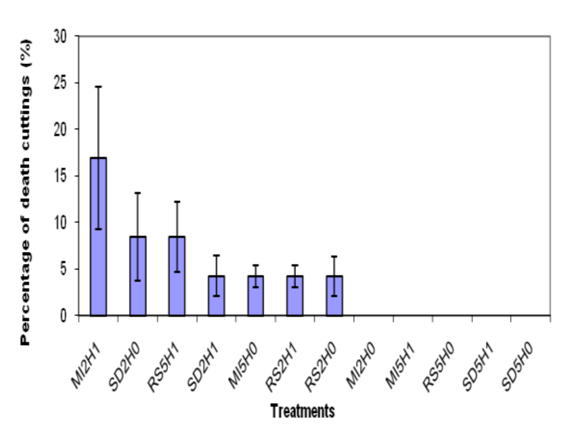

Fig.1. Effect of cutting treatments on mortality of cuttings (MI = mixture sand and sawdust, SD = sawdust, RS = river sand, 2 and 5 are the lengths, H0 = not treated and H1 = treated with seradix-2).

Mortality rate of D. crassiflora cutting

The mortality rate of *D. crassiflora* stem cuttings under the 10 different treatment combinations was assessed at the 11th week following setting up of stem cuttings in the non mist propagator, using a logistic regression model and interactions considers as independent variables. The accumulated analysis of deviance (Table 4) indicates that apart from the Length (in spite of the probability of 0.06, very closed to 0.05) and the three way interaction between rooting medium, cutting length and auxin treatment (*P=0.035*), no other treatment effect (simple or two-way interaction) significantly (*P=0.05*) contributed to the mortality of *D. crassiflora* cuttings (Fig. 1). The cuttings of 2 cm length died more than those of 5 cm with an average of 6.34 ± 3.3 % and 2.11 ± 1.0 % respectively.

Table 5. Treatment combination and average number of roots per rooted of *D. crassiflora*.

Factors		Not treated with Seradix-2		Treated with Seradix-2	
Substrate	Length	Number of roots	s.e.	Number of roots	s.e.
Mixed	2cm	1.33	0.66	1.16	0.62
	5cm	1.23	0.64	1.16	0.62
Sand	2cm	1.27	0.82	1.35	0.85
	5cm	1.00	0.72	1.10	0.61
Sawdust	2cm	1.16	0.62	1.47	0.70
	5cm	1.06	0.83	1.30	0.82

Table 6. Accumulated analysis of deviance of average number of roots per rooted cutting of *D. crassiflora*.

Sources of variation	d.f.	Deviance	Mean deviance	Deviance ration	Approx chi probability
Block	2	0.09	0.05	0.05	0.96
Rooting medium	2	0.05	0.03	0.03	0.98
Length	1	0.08	0.08	0.08	0.78
Auxin	1	0.02	0.02	0.02	0.88
Rooting medium *length	2	0.05	0.02	0.02	0.98
Rooting medium *Auxin	2	0.17	0.08	0.08	0.92
Length*Auxin	1	0.00	0.00	0.00	0.96
Rooting medium *length*Auxin	2	0.00	0.00	0.00	1.00
Residual	16	1.11	0.07		
Total	29	1.57	0.05		

Average number of roots per rooted cuttings of D. crassiflora

The number of roots per rooted cutting varied from 1 to 3. However, the mean number computed as the total number of roots developed divided by the number of stem cuttings that effectively rooted per treatment combination (Table 5) was around 1. Results of the analysis of deviance after fitting the log linear model to the observed data showed that none of the 3 factors under investigation or their interactions had a significant ($p > 0.05$) effect on the number of roots developed by *D. crassiflora* cuttings (Table 6).

Discussion

Rooting of *D. crassiflora* stem cuttings occurred 9 to 11 weeks after setting of stem cuttings in the non mist propagator. This latent period seems very long compared to cuttings of *Khaya ivorensis, Lovoa trichilioïdes and R. heudelotti*, which start to produce roots after 2 weeks (Schiembo *et al.*, 1997; Tchoundjeu, 1989). Apart from genetic differences between the species (Aparicio *et al.* 2009), this longer latent period may be explained by the difference in wood density. In fact, although all three are timber species, *D. crassiflora* has the greatest wood air dry density (1 g/cm^3) compared to *Khaya ivorensis* (0.57 g/m^3) and *Lovoa trichilioïdes* (0.50 g/cm^3). According Leakey (2004), Dick and Leakey (2006), juvenile stage material has more aptitude to develop roots than mature materials. It is well known that plant with physiologically mature materials have highest density, then have less aptitude to develop rapidly roots.

The percentage of rooted *D. crassiflora* stem cuttings was low compared to more than 80 % observed for *Dacryodes edulis* (Mialoundama *et al.*, 2002) and *Pausinystalia johimbe* (Ngo Mpeck *et al.*, 2003). Lower auxin concentration could influence rooting ability of *D. crassiflora* stem cuttings because increase rooting rates have been reported in *Dacryodes edulis* (Mialoundama *et al.*, 2002) and *Pausinystalia johimbe* (Ngo Mpeck *et al.*, 2003). Although, Seradix-2 is well known as an exogenous IBA (4 – indol – 3 yl butyric acid auxin), is generally known for adventitious roots stimulation, it however, contains only 0.3% of IBA. Probably, increasing the IBA auxin concentration could result in a corresponding increase in the percentage of rooted stem cuttings of *D. crassiflora* as observed in *P. johimbe* (Ngo Mpeck *et al.* 2004).

Rooting media also significantly increased the percentage of rooted stem cuttings. Similar observations have been reported for rooted stem cuttings of other species [*Gnetum africanum* (Shiembo *et al,* 1996; *Ricinodendron heudelotii* (Shiembo *et al.*, 1997); *Dacryodes edulis* (Mialoundama *et al.,* 2002); *Allanlackia floribunda* (Atangana *et al.*, 2006); *Nauclea diderrichii* (Caspa *et al.*, 2009)]. The best substrate for *D. crassiflora* was sawdust with more than 41% of rooted stem cuttings. This result is consistent with those obtained by Shiembo *et al.* (1996, 1997) for *Gnetum africanum* and *Ricinodendron heudelotii*. However, for other species the mixture of sand + decomposed sawdust (Mialoundama *et al.*, 2002; Atangana *et al.,* 2006) or fine sand (Caspa *et al.,* 2009) produced the highest rooting percentages. The reasons why different species display contrasting rooting percentages in different rooting media are not well understood (Leakey *et al.,* 1990) but may be attributed to variation in the oxygen, water content, pH and porosity of the media. These factors may affect tissue respiration and cell differentiation at the base of the cutting, and subsequent root development (Leakey *et al.*, 1994). In this experiment, the relatively high rooting percentage recorded in sawdust may be attributed to its relatively high air: water ratio and water content (Shiembo *et al.*, 1996).

Length of *D. crassiflora* cuttings had no significant effect on the rooting percentage. However, better rooting was observed on longer cuttings, but the differences were not significant. In addition, at week 11 the percentage of mortality was highest with shorter cuttings. The same tendency was reported by Ngo Mpeck *et al.* (2003) on *P. johimbe*, Mialoundama *et al.* (2002) on *D. edulis* and Atangana *et al.* (2006) on *A. floribunda*. The influence of cutting length on rooting ability might be due to the quantity of carbohydrates stocked in the cells. Before root initiation, the cuttings rely on the reserve of carbohydrates stored in their stem tissues. Consequently, one would expect that longer cuttings store more carbohydrates and auxins, and therefore better rooting performance. The higher mortality of stem cuttings of 2 cm length could be explained by lower reserves of carbohydrate and the tenderness of the stem tissues, compared with cuttings with 5 cm (Brian and Nina, 1988).

Exogenic auxin application at the base of cuttings promoted root development of stem cuttings at week 11, and has been attributed to enhanced transport of carbohydrate to the base of the cuttings (Hartmann *et al.*, 1990). However, others studies have reported contrasting effects of auxin application on rooting percentage. The result from this experiment is consistent with those reported for *Prunus africana* (Tchoundjeu *et al.*, 2002), *R. heudelotii* (Ngo Mpeck *et al.*, 2004). In these cases, the final rooting percentage was significantly increased by auxin treatments. It thus seems that application of IBA augments the level of endogenous auxins resulting in the full expression of rooting ability (proportion of cutting rooting and number of roots develop per rooted cutting). However, others have reported no improvement in the final rooting of cuttings treated with IBA, e.g. *Shorea leprosula* (Aminah *et al.*, 1995); *I. gabonensis* (Shiembo *et al.*, 1996); *R. heudelotii* (Shiembo *et al.*, 1997) and Miombo fruits trees (Akinnifesi *et al.,* 2008). The contrary observed in this case could be explained by the physiology and genetic makeup of the different species as described by Akinnifesi *et al.* (2008).

Conclusion

This study demonstrates that *D. crassiflora* can be effectively propagated by stem cuttings from young twigs. Root development occurs between 9 and 11 weeks after setting of stem cuttings in the non mist propagator. Based on this experiment, the best rooting media are either decomposed sawdust or mixed with river sand. The maximum number of rooted cuttings (61 %) was obtained 11 weeks after setting stem cuttings in non mist propagators. The rooting rate was marginally increased when stem cuttings were pretreated with root enhancing auxin (Seradix-2). Under these conditions, longer stem cuttings (5 cm) performed better than shorter (2 cm) ones and the percentage of rooted stem cuttings rose to more than 60% of the total number set in the non mist propagator. Furthermore, the average number of roots per rooted stem cutting was appreciable. Research assessing the effects of various auxin concentrations and types on rooting of *D. crassiflora* is further warranted as a logical follow up to this study.

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