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Full length Research Paper

Comparison between field performance of cuttings and seedlings of *Carapa procera* D.C. (Meliaceae)

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The use of vegetative propagules of *Carapa procera* has been an important tool for the large scale deployment of improved plants. However, given the reported morphological differences in root systems between cuttings and seedlings, the question of whether such differences affect growth and wood quality needs to be addressed. The present study compares growth (diameter and height) and wood density (pilodyn penetration) of vegetatively propagated cuttings and seedlings from the same or related pedigrees. The relevance of age, site and the interaction between propagation method and genetic improvement were also investigated. Trials included full-sib progenies, in which each progeny was tested as cuttings and seedlings, and progeny trials where parents were cloned and offspring derived from open pollinated crosses. The results show that there were no significant differences between the two types of plant material (cuttings versus seedlings) for the traits examined in the study.

Keywords: Seedling, cutting, growth, wood density, *Carapa procera.*

INTRODUCTION

The aim of any improvement programme is to deploy the best genetically improved plants, as effectively and extensively as possible, either by seed or through vegetative propagation. The early years of tree breeding provided few examples of clonal deployment (Lambeth et al., 1994), but many programs around the world today rely on vegetative propagation (Shelbourne and Thulin, 1974). This is particularly the case with tropical trees species, where cloning constitutes a valuable tool in most improvement programs (CTFT, 1989; Teillier, 1994). With tropical tree species, such as *Carapa procera*, the use of cuttings has been hampered by propagation constraints such as low rooting ability, although such problems have also been recently overcome (Teillier, 1994; Bongou and Wilson, 1994). One issue concerning clone deployment is the negative impact of propagation effects. There are several studies comparing growth between cuttings and seedlings in forest trees (Foster et al., 1987; Frampton, 1993; Teillier, 1994), but only a few deal with Carapa (Cornuet and Luikart, 1997; Dayanandan et al., 1999). Such comparisons often use seedlings and cuttings of different genetic backgrounds, thus complicating the interpretation of the results (Sweet and Wells, 1974). In general, differences in field performance have not been apparent. Furthermore, some studies revealed that such differences tend to decrease over time as the trees mature (Gemmel et al., 1991; Goudet et al., 1996; Goudet, 2002). Therefore, caution should be exercised when the studies are conducted in young trees.

In the case of *C. procera*, previous studies comparing root characteristics of seedlings and cuttings have yielded considerable evidence that differences in the root system exist between the two propagation types, with cuttings producing fewer primary roots, often with no tap root, and having a shallower root system (Delwaulle, 1996). Additional studies comparing the growth of cuttings and seedlings of *C. procera* in the field (Delwaulle, 1996; Pangou et al., 2011) have shown that the deformation observed in the root systems of cuttings

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may reduce their functional effectiveness and can affect the growth of cuttings.

Some authors argue that such differences could explain the slower initial growth of cuttings compared with seedlings of similar genetic background (Foster, 1985; Foster et al., 1987. Struve, 1984). However, a common weakness in most of those studies lies in the differing genetic backgrounds of seedlings and cuttings. In addition, most of these studies have not been followed over time. The aim of the present research study was to compare field performance between seedlings and cuttings in terms of growth and wood density (pilodyn penetration) in *C. procera* field trials up to an age closer to full rotation.

MATERIALS AND METHODS

Plant material

The genetic material included in this study comprises a group of plus-trees (parents), originally selected in 8–12 year-old commercial plantations in Congo, on the basis of overall good growth and form. Cuttings and open pollinated seed were collected from them and subsequently used to establish field trials. From some of these plus trees, grafts and controlled crosses were made. These parents are commonly referred to as belonging to the Congolese Northern Rainforest Provenance.

Forest tree breeding for fruit and seed production is being increasingly implemented in the humid tropics of Africa as part of the domestication of high-value indigenous multi-purpose trees (Delwaulle, 1995; Leakey and Simons, 1998; Leakey et al., 2000; Tchoundjeu et al., 2006). Candidate species that has been targeted for improvement include *C. procera* D.C., commonly known as tallow-tree, which is a member of the Meliaceae family. Mature *C. procera* is a medium sized tree species, about 30 m in height and 80 cm in diameter at breast height, which is found in South America in Surinam, French Guiana and the State of Amazonas in Brazil, as well as in Western and Central Africa. Tallow-tree occurs in mixed stands in evergreen lowland and deciduous forests, which have two wet and two dry seasons, and an annual rainfall ranging between 1900 and 4000 mm (Maitre, 986; CTFT, 1989). In Congo, this monoecious species flowers from December to January, and produces fruit from March to July, with many possible variations from year to year (Pangou, 2007). The bark of *C. procera* is locally used against coughs, dysentery, diarrhea, toothache, and as an aphrodisiac and pain reliever. Moreover, its seeds are rich in a hard white fat (67-73%) consisting mostly of stearic and oleic acids (Foma and Abdala, 1985), hence the species' common name. Oleic and stearic acids are reported to lower plasma cholesterol levels (Bonanome and Grundy, 1988), thus reducing the risks of heart attack. Owing to this profile, *C. procera* seed fat is used for margarine production and in the manufacture of soap and ointments. The seeds that are extracted from *C. procera* fruits by local communities are purchased by local and foreign agro-industries.

The companies have created a guaranteed market for the product, which is expected to grow into a new industry for Africa. Currently, attempts to improve *C. procera* trees have been restricted to the development of appropriate protocols for rooting of leafy stem cuttings (Bibila et al., 2006), and to surveys of clonal variation in the rooting of cuttings (Bibila and Phaka, 2008). The germination rate of *C. procera* seeds is very high (more than 60% (Pangou et al., 2007).

Field trials

In the study two sets of trials were used. The first set (denoted here as the full sib trials) was established in two locations (Table 1). Each trial included several mostly unrelated full-sib families derived from controlled crosses between plus trees, and whose progeny was tested as both seedlings and cuttings.

The second set of trials (denoted the Open Pollinated Trials) was established in seven locations (Table 1), and included the parent (the original plus tree), propagated as cuttings and their open pollinated offspring deployed as seedlings.

Full sib trials

The plants used in these trials were obtained from controlled crosses, carried out amongst grafted plus trees in the seed orchards of GERDIB (Biological Diversity Study and Research Group). Crosses were mostly unrelated although some families may share a common parent. No reciprocals and selfs were included. The seeds obtained from these crosses were divided into two lots. The first lot was raised in containers and cloned according to standard macro-propagation procedures (Babin and Butcher, 1995). Cuttings, typically with one leaf pair and 10 cm long, were dipped into hormone powder, and set in a medium composed of 60% peat and 40% styrofoam and set to root in a glasshouse. Rooting success varied amongst families and was generally low (around 20%), as expected from *C. procera* material. The second seed lot was kept apart and only set to germinate at a later stage, as to ensure cuttings and seedlings were of similar size at planting.

These trials allowed a direct comparison between plants of the same genetic background (full sib progeny) but propagated by different means (cuttings versus seedlings).

Table 1. Details of age, climate and location of field trials used in this study.

Set of trials	Site	Establishment	Location	Elevation (m asl)	Mean rainfall (mm/yr)
Full sib	Impfondo (IL)	01/03/1995	18°03’E-1°93’N	350-650	2500
	Dimonika (DK)	01/12/1995	12°26’E-4°13’S	325-450	2000
Open Pollinated	Koulila	27/04/1989	12°25’E-4°10’S	325-450	2000
	Holle	14/03/1990	12°27’E-4°12’S	15-50	2000
	Bilala	23/04/1991	12°26’E-4°13’S	20-50	2000
	Enyele	27/02/1991	11°37’E-4°03’S	350-650	2500
	Etumbi	01/03/1991	18°13’E-1°23’N	275-400	2500
	Louesso	21/02/1990	17°63’E-3°33’N	444-650	2500
	Itoli	01/02/1990	17°54’E-3°23’N	335-500	2500

Table 2. Number of replicates of each family and number of families of full sib trials.

Trial		Replicates	Progenies
Impfondo (IL)	Cutting	4	40
	Seedling	12	76
Dimonika (DK)	Cutting	5	65
	Seedling	5	59

The two full sib trials were established in reasonably fertile sites in Congo, in the North East (IL trial) and South (DK trial) of the country (Table 1). The trials were established in March and December 2001, respectively, at a spacing of approximately 4 × 2 m. Establishment included a ripping, cultivation, and around 350 kg/ha of NPK (10:6:10) fertilization applied at two occasions (at age 1 and age 3).

Both sites have an irregular experimental design. Cuttings and seedlings were established in two contiguous (homogeneous) areas and replicated within each *area*. The sets of families nested within each *area* were allocated using a randomized complete block (RCB) design with single tree plots. Most families were represented as both seedlings and cuttings, although some families were only tested with one plant type due to problems related to seed availability, rooting success of cuttings and survival in the field (Table 1).

Open pollinated trials

In these trials, the parents were propagated through cuttings and were established together with their open pollinated progeny, propagated as seedlings. The original cuttings were obtained directly from the sprouts emerging from the stump after the original tree was harvested. This material derived from epicormic shoots can therefore be considered juvenile. Unlike the full sib trials, the cloned parents were expected to share only half the genetic background of their offspring, the other half being determined by the pollen contribution of unknown value. The genetic merit of the mothers is expected to be better than the average of the fathers (pollen), but only slightly, given the low efficiency of mass selection. Since families were openly pollinated, the progeny’s value is expected to be 1/2 the mother’s value; and assuming the average contribution of the parents is neutral (neither better nor worse than the average), then the parents are expected to be somewhat better (or at least the same) than their OP offspring. Otherwise it may suggest the presence of negative propagation effects.

In the open pollinated trials, the experimental design included a RCB design, with the cloned parents and their seedling progeny randomly allocated in five replicates (Table 2). Plot sizes consisted of five trees in a row. In total there were 36 treatments including 10 parents-offspring pairs and 26 progeny. The trials were established between February and March 1998, at a spacing of approximately 4 × 2 m. Establishment included

Table 3. Comparison of means (and associated *F*-test) between cuttings and seedlings and variances values for Family and Family × Method interactions (and associated LTR test) in the full sib trials at several ages and for diameter, height, and pilodyn penetration.

Variable	**Trial**	**Age (years)**	**Mean of the trial**	**[X_s – X_c]***	**†**	**Variances**		
						Families†	F × M†	Error
Height (m)	IL	2.7	8.6	1.01	***	1.13 ***	0.00 ns	2.01
		3.5	10.7	0.94	***	0.98 ns	0.00 ns	3.01
	DK	2.8	8.9	0.026	ns	1.37 ns	0.05 ns	1.72
Diameter (cm)	IL	2.7	7.4	0.48	ns	1.28 ns	0.00 ns	3.07
		3.5	8.6	0.13	ns	1.02 ns	0.00 ns	4.24
		4.4	10.1	-0.29	ns	1.05 ns	0.08 ns	5.52
	DK	2.8	7.4	0.48	ns	1.18 ns	0.12 ns	2.48
		3.5	9.1	0.15	ns	1.51 ns	0.07 ns	3.31
		5.6	12.5	-0.24	ns	1.82 **	0.04 ns	6.39
Pilodyn (mm)	IL	4.4	21.7	-0328	ns	0.94 ns	0.78 *	3.72
	DK	3.7	20.4	0.63	ns	1.26 ns	0.00 ns	3.34

*: X_s mean of seedlings; X_c mean of rooted cuttings; † ns: not significant at $p = 0.05$; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

a ripping and cultivation, fertilized once with an NPK (10:6:10) fertilization between ages 1 and 3.

Measurements and analysis

Trees were measured for height (h) in meters, diameter at 1.30 m (d) in cm and pilodyn penetration (P) in mm (Greaves et al., 1996). The statistical analysis for the full sib trials used the linear model:

$Y_{ijkl} = \mu + F_i + M_j + Z_k + FM_{ij} + \varepsilon_{ijkl.}$ (1)

The statistical analysis of the open pollinated trials used the linear model:

$Y_{ijkl} = \mu + F_i + B_b + M_j j + FM_{ij} + \varepsilon_{ibjl}$ (2)

where Y represents the value of each individual for the respective characteristic; μ represents the overall mean of the trial; F_i represents the effect of the ith family, considered random; M_j represents the effect of the j_{th} method of the propagation, considered fixed; B_b represents the effect of the bth block, considered fixed; Z_k represents the effect of the kth replicate, considered fixed; FM_{ij} represents the effect of the interaction between the ith family and the jth method, considered random; ε_{ibjl} represents the residual.

All analyses were carried out using the programme ASREML (Gilmour et al., 1996). To estimate the significance of random effects, a LRT (likelihood ratio test) test was carried out (with a single degree of freedom). The test performed to evaluate the significance of fixed effects, namely between propagation methods, was based on Wald's *F* test.

RESULTS

Table 3 shows that seedling material had significant greater height in one trial (IL), although differences were greater at age 2.7 years than at age 3.5 years. In the second trial (DK), at 2.8 years, there was no difference in height between seedlings and cuttings.

Diameter results were more comparable across the two sites. In trial IL, seedlings had initially greater diameter than cuttings at age 2.7 years, but by age 4.4 years this was reversed. In trial DK, the seedling's diameter was also greater than cuttings at 2.8 years, but at 5.8 years the diameter of cuttings was greater than that of seedlings. In all cases, differences between the two plant types were not significant.

A similar conclusion could be drawn for pilodyn penetration. Measurements taken at 4 years in trials IL and DK have yielded no significant differences between treatments.

The results, therefore, suggest a slightly better initial development for seedlings (up to age 2) but otherwise a similar growth between cuttings and seedlings. At age 4 or 5 years, growth seems to be similar between cuttings and seedlings of similar genetic background.

In the open pollinated series of trials, the results provided further evidence of small propagation effects in growth. In six out of the seven trials measured for diameter, the cloned parents grew faster than their seedling open pollinated progeny, although only three were statistically significant. The ages ranged between 5 and 9 years old. The difference in pilodyn penetration, measured only in one site, was again not significant. The lack of significant difference is also an expected result since initial selection of the parents was based on growth and not wood density, and the two traits are mostly unrelated.

Although the study was not intended to measure the magnitude of genetic effects in this material, some conclusions can be drawn from the analysis of variance. In the full sib series of trials, progeny effects were only

Table 4. Comparison of means (and associated *F*-test) between cuttings and seedlings and variances values for family and clones (and associated LTR test) in the open pollinated trials, for the variables diameter, height and pilodyn penetration.

Variable	Trial	Age (years)	Mean of the trial	$[X_s – X_c]$*	†	Variances		
						Families/clones	F × M†	Error
Height (m)	Dimonika	4.6	8.6	2.52	***	3.98	***	2.190
	Holle	5.8	10.9	–2.86	***	2.52	***	3.089
	Impfondo	8.2	12.7	–1.00	***	2.56	***	3.242
	Enyele	8.6	16.4	–0.11	ns	2.37	***	2.053
Diameter (cm)	Etumbi	4.6	8.6	–0.50	*	3.21	***	3.664
	Dimonika	5.8	10.9	–1.58	**	1.31	ns	7.844
	Impfondo	7.2	14.5	0.37	ns	3.31	***	11.352
	Holle	7.6	13.7	–0.39	ns	1.88	*	3.861
	Bilala	8.2	12.7	–1.19	ns	2.19	*	6.380
	Enyele	8.6	16.4	–0.81	ns	2.40	***	4.175
	Etumbi	9.4	14.1	–1.56	**	1.96	*	7.664
Pilodyn (mm)	Enyele	8.6	21.1	0.30	ns	5.47	***	1.064

*: X_s mean of seedlings; X_c mean of rooted cuttings; † ns: not significant at $p = 0.05$; * significant at $p < 0.05$; ** significant at $p < 0.01$; *** significant at $p < 0.001$.

significant at later stages of development on one of the sites (DK), thus suggesting that genetic variation for growth is low. Interaction between family and propagation method (for height and diameter) was also not significant. At age 4.4 years in trial IL, interaction effects for pilodyn penetration was significant at a 5% probability level, but in this particular case, family effects were not significant (Table 3).

However, this lack of a statistically significant family effect may be exacerbated because some of the full sib families involved are related (some parents are used in more than one cross), hence the observed family differences are less than the expected half of the (additive) genetic variation in the population. In the open pollinated series of trials, family effects (expected to represent 1/3 to 1/4 of the additive genetic variance) were generally larger and significant (Table 4).

DISCUSSION

The results observed here suggest that seedlings and cuttings of *C. procera* of similar genetic background had similar growth rates and wood density. In the full sib series of trials, there was some evidence that initial growth was greater in seedlings up to age 2, but by age 4 or 5 these differences either disappeared or were significantly reduced. These initial differences may be more the result of poor plant quality of the cuttings, than ontogenetic effects. The present study did not include any assessment of root characteristics; however, clear differences in the structure of root systems between cuttings and seedlings of *C. procera* have been reported for this species by Teillier (1994) and Delwaulle (1996). These authors reported more deformities and less radial symmetry in the root system of cuttings and concluded that such malformations are negatively correlated with initial height growth. Some authors have claimed that rooted cuttings may be at a different stage of maturation, hence leading to ontogenic differences in growth and morphology (Foster et al., 1985; Greenwood, 1984; Vigneron, 1984; Stelzer et al., 1998; Sweet, 1973). Whereas this may be the case with conifers, it is less likely to apply to *C. procera*, which regenerates from coppice derived from juvenile epicormic buds. No apparent morphological differences could be found between the various materials tested.

Similar results were obtained in the present study with the open pollinated series of trials. They found no evidence of significant growth differences between cloned parents and their open pollinated progeny seedlings. In fact, clones were found to perform slightly better. While this is an expected result (since genetic merit of the progeny is expected to be only 1/2 of the merit of the selected female parent), it at least suggests that propagation effects were not likely to have affected the parent's performance significantly.

Results reported in the literature have been contradictory. Teillier (1994) reported that unimproved *C. procera* seedlings presented similar and in some cases, greater growth than first generation selected cuttings, suggesting therefore a reduction in growth due to cloning effects. In a *Baillonella toxisperma* trial similar to our open pollinated series, Bongou and Wilson (1994) also reported poorer growth in the cloned parents in comparison with their open pollinated progeny seedlings. On the other hand, Lambeth et al. (1994), in *Eucalytus grandis* and Vigneron (1984) and Paul et al. (1993) in

Terminalia superb, found a clear superiority of improved cuttings over unimproved seedlings. Most of these studies are inadequate because materials are not comparable (Delwaulle, 1995). Nevertheless, the disparity of results between studies highlights the importance of plant quality of cuttings as a critical factor, at least during initial stages of development (Foster, 1987). In the *C. procera*, a species known as difficult to root, plant quality issues must play an important role. For the plant material tested here, both series of trials support the idea that no unfavorable propagation effects were associated with cuttings, and there were no reductions in performance between seedlings and cuttings of similar genetic background.

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