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Field growth performances of teak genotypes of different ages clonally produced by rooted cuttings, in vitro microcuttings, and meristem culture

Olivier Monteuuis and Doreen Kim Soh Goh

Abstract: Teak (*Tectona grandis* L. f.) clonal forestry has lately become a reality thanks to the development of efficient techniques for mass clonally propagating true-to-type teak trees of various ages. Field trials were set up to assess the influence of teak genotypes of different ages and three clonal propagation techniques on field growth performances of teak clones. Significant differences ($P < 0.0001$) in height (H, from 11.9 to 17.5 m), diameter at breast height (D, from 11.8 to 18.9 cm), and volume (V, from 67.9 to 194.7 dm^3) were observed 5 years after planting for clones produced by microcuttings from 6-month-old to 70-year-old teak ortets, regardless of their age. After 6.5 years of testing, H, D, and V performances of clones produced by rooted cuttings and microcuttings from 7-year-old teak trees were similar, notwithstanding clone × propagation method interactions. Five years after planting, clones produced by meristem culture from 7-year-old ortets had larger diameters and volumes than clones produced by microcuttings, whereas H varied according to clone × propagation method interaction. The various propagation methods used had no significant effect on mortality (<10%). The pros and cons of these techniques for mass clonally propagating teak genotypes of different ages were discussed.

Key words: Tectona grandis, clonal propagation methods, growth performances, age, clone.

Résumé : La foresterie clonale du teck (*Tectona grandis* L. f.) est récemment devenue une réalité grâce à la mise au point de techniques performantes permettant le clonage conforme de tecks plus ou moins âgés. Quatre essais au champ ont été mis en place pour analyser l'influence de génotypes d'âges variables et de trois techniques de propagation clonale sur la croissance au champ de clones de teck. Des différences significatives ($P < 0.0001$) de hauteur (H, de 11,9 à 17,5 m), de diamètre à hauteur de poitrine (D, de 11,8 à 18.9 cm), et de volume (V, de 67,9 à 194,7 dm^3) ont été constatées 5 ans après plantation pour des clones produits par microbouturage à partir d'ortets âgés de 6 mois à 70 ans, indépendamment de leur âge. A 6,5 ans, les valeurs de H, D, et V étaient similaires entre des plants issus de bouturage et de microbouturage d'individus âgés de 7 ans, avec de fortes interactions clone × technique de propagation. Après 5 ans de plantation, les clones produits par culture de méristème d'ortets de 7 ans avaient des diamètres et des volumes supérieurs à leurs homologues issus de microbouturage, la hauteur variant en fonction des interactions clone × technique de clonage. Aucune différence significative de mortalité (<10 %) n'a été constatée entre les techniques de propagation testées. Les avantages et inconvénients respectifs de ces techniques pour cloner à grande échelle des génotypes de tecks d'âges variables sont discutés.

Mots-clés : Tectona grandis, techniques de propagation clonale, croissance, âge, clone.

Introduction

Tectona grandis L. f., commonly known as teak and belonging to the Lamiaceae family, is a large, long-lived tree species native of India, Laos, Myanmar (ex Burma), and Thailand (White 1991; Tewari 1992). Its worldwide reputation is due to the outstanding properties of its wood, with a special mention for its durability and aesthetic features (Kadambi 1972; Food and Agriculture Organization of the United Nations (FAO) 2009). This attractiveness has spurred the introduction of the species for timber production in several tropical countries of Asia, starting with Indonesia some four to six centuries ago (Siswamartana 2000; Verhaegen et al. 2010), and then it was introduced to Africa and Latin America (Ball et al. 2000). The list of countries that have embarked on industrial teak plantations has rapidly expanded lately under the impulse of private investors eager to meet the increasing demand for high-grade timber in the wake of declining supplies from natural stands (FAO 2009; Kollert and Cherubini 2012). These planting activities have been promoted recently by the possibility to mass produce selected teak clones, far superior in regards to timber yield, quality, and uniformity to the seedlings or stumps traditionally used for establishing teak plantations (Goh and Monteuuis 2012; De Camino and Morales 2013; Ugalde Arias 2013). For teak, the use of planting material produced from seeds is impeded by several limitations, in particular, insufficient fruit production, low germination rates, and a disadvantageous positive correlation between flowering age and forking height (White 1991; Kaosa-ard et al. 1998; Callister 2013). Seed-derived teak trees, even from the same progeny, also show substantial variability for economically important traits, these appearing to be mainly under nonadditive control (Kjaer et al. 2000; Callister and Collins 2008; Chaix et al. 2011; Monteuuis et al. 2011). These limitations have warranted decades long efforts for developing alternative strategies, focusing on efficient methods for mass

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O. Monteuuis. CIRAD – BIOS Department – UMR AGAP, TA A-108/03 – Avenue Agropolis, F-34398 Montpellier, Cedex 5, France.
D.K.S. Goh. YSG Biotech Sdn Bhd, Yayasan Sabah Group, Voluntary Association Complex, mile 2 ½, off Tuaran Road, P.O. Box 11623, 88817 Kota Kinabalu, Sabah, Malaysia.
Corresponding author: Olivier Monteuuis (e-mail: olivier.monteuuis@cirad.fr).

Table 1. Characteristics of the 18 teak ortets from which the clones were derived.

Genotype	Location of ortets	Age	Additional information
1–8	Luasong Forestry Center, Tawau district, Sabah, East Malaysia	7 years	Derived Solomon Islands seed source, presumably from a natural provenance: Myanmar or ex Burma (Goh and Monteuuis 2009, 2012)
9–11	Luasong Forestry Center, Tawau district, Sabah, East Malaysia	6 months	Seed presumably from Thailand
12–16	Mata Ayer, state of Perlis, West Malaysia	30–45 years	Situation: 75°29'E. Derived from a seed of uncertain origin (Krishnapillay and Abdul Razak Mohd Ali 2000)
17	Jalan Apas, Tawau district, Sabah, East Malaysia	27 years	Derived from a seed imported from Trinidad (Lapongan 2000)
18	Bandau, Kota Marudu district, Sabah, East Malaysia	65–70 years	Planted by the Dutch Tobacco Company in 1926 or 1929 (Lapongan 2000)

Note: The age is the presumed age of the ortets at the time of explant collection for in vitro cloning.

producing superior teak clones from mature selected plus trees. Due to the inhibitory effect of ageing on adventitious rooting (Bonga 1982; Ahuja and Libby 1993; Husen and Pal 2006), clonal propagation of mature selected teak trees has hitherto been restricted to the use of grafting and budding mainly for establishing clonal seed orchards (Kaosa-ard et al. 1998). For the first time, Gupta et al. (1980) reported the possibility to micropropagate by axillary budding in vitro juvenile and even 100-year-old teak genotypes. However, this success remained limited to an experimental scale. Mass production of tissue-cultured teak plants, mostly from juvenile seedlings, started in Thailand, but these activities were hindered by high production costs (Kaosa-ard et al. 1998). Most of the commercial clones currently planted were derived from protocols developed in Sabah, East Malaysia, in the early 1990s for efficiently mass cloning true-to-type teak plus trees of various ages (Goh and Monteuuis 2012; Ugalde Arias 2013). These protocols encompassed propagation by rooted cuttings (Monteuuis 1995; Monteuuis et al. 1995) and by in vitro microcuttings and meristem culture (Monteuuis et al. 1998). It can be assumed for teak, as well as for many tree species, that field performances of clones are liable to vary according to the age and to the genotype of the mother tree, or ortet, initially selected, as age effects are genotype related (Bonga 1982; Monteuuis 1985; Ahuja and Libby 1993). The clonal propagation methods used can also interact (Hartmann et al. 1997). This was practically assessed by comparing the height, diameter, and volume scores of field-established teak clones produced in vitro by microcuttings from ortets of different ages. The influence of the genotype and of the propagation methods used, i.e., rooted cuttings vs. in vitro microcuttings vs. meristem culture, was more closely investigated 5 and 6.5 years after planting by measuring and comparing the height, diameter, and volume of clones derived from 7-year-old ortets.

Materials and methods

Plant material and propagation methods

The characteristics of the 18 seed-derived and various aged teak genotypes used for the experiments are detailed in Table 1. The clones were produced from the mother trees by three different vegetative propagation methods. The rooted cuttings were obtained after three cycles of serial propagation in suitable nursery conditions by applying the procedure detailed in Monteuuis (1995) and Monteuuis et al. (1995). The microcuttings were initiated from 1 to 2 cm long nodal explants and were serially micropropagated in vitro by axillary budding as described in Monteuuis et al. (1998). After 2 years of repeated subcultures, the 3 to 4 cm tall microshoots were transferred to suitable ex vitro conditions, i.e., in sand beds under shade and intermittent misting, where 90%–100% of the microshoots rooted after 3–4 weeks, in accordance with Bonal and Monteuuis (1997). Shoot apical meristem (SAM) culture was similar to the microcutting procedure except for the initial stage, where 0.1 to 0.3 mm long SAMs were used as primary explants (Monteuuis et al. 1998). Once rooted ex vitro, the cuttings and microcuttings were potted individually in 10cm × 15 cm black plastic bags filled with local clayey topsoil that was mixed with sand for better drainage. After a raising period of 3 months, the plants reached the suitable height of 20–30 cm for field planting. All the plant materials compared were rigorously produced in the same conditions and were of the same size when they were field planted.

Trial characteristics and relevant statistical models

Four different and independent field trials were established to assess the influence of the experimental factors investigated on field growth. These trials were set up in Taliwas, at km 18 on the road from Silam to Danum Valley, Sabah, East Malaysia (latitude 4°58′N, longitude 118°13′E). Monthly temperatures were 26–28 °C, and mean annual rainfall averaged 2500 mm without a distinct dry season. The other site characteristics are detailed in Chaix et al. (2011). Most of the planting area was flat, on the bottom of a valley at 40–60 m above sea level, close to a river, and prone to short periods of flooding, which necessitated the creation of ditches in some places within and on the periphery of the trial. The site was prepared by ripping and mounding just before planting. A randomized complete block (RCB) design was adopted for the four field trials, with one row plot replicate of each treatment per block. The number of blocks and trees per row plot varied according to the trials. The spacing was 4 m between rows and 2 m within rows.

Trial 1: genotypes of different ages

The growth performances 5 years after the planting of clones produced by microcuttings from the 18 different-aged teak ortets were compared. The layout consisted of three blocks, the 18 clones being represented in each block by one row plot of 8 to 12 trees per plot. The following statistical model was used:

$$(1) \qquad Y_{ijk} = \mu + C_i + R_j + (\mathrm{CR})_{ij} + \varepsilon_{ijk}$$

where the variables are as follows: Y_{ijk}, the observation on the kth individual of the ith clone in the jth block; μ, overall mean; C_i, effect of the factor "clone", $1 \le i \le 18$; R_j, effect of the factor "replicate", $1 \le j \le 3$; $(\mathrm{CR})_{ij}$, effect of the interaction between clone and replicate factors; and ε_{ijk}, residual error.

Trial 2: genotype and cuttings vs. microcuttings

The growth performances 6.5 years after the planting of cutting-derived clones was compared with the growth performances of microcutting-derived clones from the 7-year-old genotypes 3, 7, and 8. Each clone × propagation method (rooted cutting or microcutting) treatment was represented by 7–12 trees per row plot within each of the five blocks of the RCB design (five replicates, one per block). The following statistical model was used:

$$(2) \qquad Y_{ijkl} = \mu + C_i + M_j + R_k + (\mathrm{CM})_{ij} + (\mathrm{CR})_{ik} + (\mathrm{MR})_{jk} + (\mathrm{MCR})_{ijk} + \varepsilon_{ijkl}$$

Table 2. Height (*H*), diameter at breast height (*D*), and volume (*V*) mean values ± standard error (SE) and coefficients of variation (CV) 5 years after planting of the 18 clones produced by microcuttings from ortets of various ages in trial 1, with the relevant number of observations (*N*) presented.

			H		*D*		*V*	
Clones	Ortet age	*N*	Mean±SE (m)	CV (%)	Mean±SE (cm)	CV (%)	Mean±SE (dm³)	CV (%)
1	7 years	32	15.9±0.4 abc	13.7	15.5±0.6 abc	20.9	123.9±9.2 bc	42.1
2	7 years	29	15.0±0.6 cde	20.7	15.1±0.7 bc	26.0	116.6±11.6 bc	53.6
3	7 years	29	14.7±0.5 cde	18.3	16.2±0.6 abc	20.5	129.6±12.1 b	50.2
4	7 years	29	14.1±0.5 cde	20.4	17.7±0.8 abc	24.1	151.5±15.5 ab	55.1
5	7 years	29	14.2±0.5 cde	20.7	16.4±0.7 abc	24.7	131.1±12.1 b	49.7
6	7 years	30	14.3±0.5 cde	18.6	15.8±0.6 abc	22.7	121.5±11.7 bc	52.6
7	7 years	29	17.5±0.4 a	13.9	17.8±0.7 abc	20.8	178.2±14.4 ab	43.4
8	7 years	25	16.0±0.5 abc	14.4	16.7±0.8 abc	25.2	150.8±17.8 ab	59.1
9	6 months	28	13.7±0.7 cdef	26.1	15.4±1.1 abc	37.7	130.1±19.1 b	77.5
10	6 months	27	14.8±0.5 cde	19.0	14.9±0.7 c	23.7	110.9±11.7 bc	54.7
11	6 months	28	13.0±0.7 ef	27.4	15.2±0.9 bc	33.3	114.3±16.7 bc	77.3
12	30–45 years	26	13.5±0.8 def	28.7	16.7±1.0 abc	32.0	141.6±20.8 ab	75.0
13	30–45 years	32	14.7±0.4 cde	16.7	18.9±1.0 a	28.9	178.3±17.4 ab	55.1
14	30–45 years	32	15.0±0.6 cde	22.0	17.0±0.8 abc	25.4	149.6±13.4 ab	50.7
15	30–45 years	29	17.1±0.4 ab	11.8	18.6±0.9 ab	25.3	194.7±19.2 a	53.1
16	30–45 years	22	13.7±0.7 cdef	24.3	18.2±1.2 abc	30.7	169.6±26.8 ab	74.2
17	27 years	26	11.9±0.7 f	29.1	11.8±0.8 d	35.6	67.9±13.1 c	98.3
18	65–70 years	33	15.6±0.4 bcd	11.8	17.1±0.6 abc	21.0	149.7±11.6 ab	44.5

Note: Lowercase letters distinguish means that are significantly different at $P_o = 0.05$ for each growth trait (Student–Newman–Keuls test).

where the variables are as follows: Y_{ijkl}, observation on the *l*th individual of the *i*th clone, *j*th propagation method, and *k*th replicate; μ, overall mean; C_i, effect of the factor "clone", $1 \le i \le 3$; M_j, effect of the factor "propagation method", $1 \le j \le 2$; R_k, effect of the factor "replicate", $1 \le k \le 5$; $(CM)_{ij}$, effect of the interaction between the clone and propagation method factors; $(CR)_{ik}$, effect of the interaction between the clone and replicate factors; $(MR)_{jk}$, effect of the interaction between the propagation method and replicate factors; $(MCR)_{ijk}$, effect of the interaction between the propagation method, clone, and replicate factors; and ε_{ijkl}, residual error.

The influence of the six combinations of clones and propagation methods was analysed using the same statistical model as eq. 1, but with C_i as the effect of the factor "clone × propagation method", where $1 \le i \le 6$ and $1 \le j \le 5$.

Trial 3: genotype and microcuttings vs. meristem cultures

Growth performances of plants produced by microcuttings and meristem culture from the 7-year-old genotypes 1, 3, and 5 were compared 5 years after planting. Each clone × propagation method (microcutting or meristem culture) combination was represented by 10–15 trees per row plot within each of the three blocks of the RCB design (three replicates, one per block). The statistical models used were as eq. 2 but with $1 \le k \le 3$, and eq. 1, with C_i as the effect of the factor "clone × propagation method", where $1 \le i \le 6$.

Trial 4: mericlones

The growth performances of two subclonal lines derived from one single shoot apical meristem, or "mericlones" (Monteuuis and Goh 1999), from clone 3 were compared 5 years after planting, each mericlone being represented in each block of the four RCB layout by one row plot of 10 trees. The statistical model eq. 1 was used for the analyses, with C_i as the effect of the factor "mericlone", where $1 \le i \le 2$ and $1 \le j \le 4$.

Traits recorded and statistical analyses

The quantitative data recorded at the respective assessment dates were

1. mortality rate, *M* (%), determined as the numbers of dead trees and expressed as a percentage of the total number of trees initially planted;
2. total tree height, *H* (in m), measured with a graduated pole and then with a clinometer when trees became too tall;
3. diameter at breast height, *D* (in cm), converted from the girth measured with a tape at about 1.30 m above soil level;
4. bole volume, *V* (in dm³), calculated by using the following formula:

$$V = \{[\pi \times (D/2)^2 \times 1.3] + [\pi \times (D/2)^2 \times (H - 1.3)/3]\}/10$$

The results are expressed as means. The coefficients of variation (CVs) associated reflect the within-sample variation. The statistical analyses were performed using the SAS statistical package, version 9.2 (SAS Institute Inc. 2008). Bartlett's test was used for checking variance homogeneity (Sokal and Rohlf 1995), and Proc GLM (SAS Institute Inc. 2008) was used for the analyses of variances according to the statistical models eq. 1 and eq. 2. The statistical significance threshold of all the statistical tests applied was set at $P_o = 0.05$. For factors with a statistically significant influence, means were compared using the Student–Newman–Keuls test (Sokal and Rohlf 1995).

Results

Mortality was less than 10% on average across the four trials and did not vary significantly according to the experimental factors investigated.

Trial 1: genotypes of different ages

Data means are reported in Table 2. The statistical analysis indicated that the 18 genotypes of various ages had a strong influence ($P < 0.0001$) on *H*, *D*, and *V* mean scores 5 years after planting, with highly significant block ($P = 0.0034$ for *H* and $P < 0.0001$ for *D* and *V*) and clone × block ($P < 0.0001$ for all three traits) effects. Clone mean values ranged from 11.9 (clone 17) to 17.5 m (clone 7) for H, from 11.8 cm (clone 17 again) to 18.9 cm (clone 13) for D, and from 67.9 dm³ (clone 17) to 194.7 dm³ (clone 15) for *V*. The comparison of *H*, *D*, and *V* mean scores and associated CV values observed for clones 1–8, 9–11, 15, and 18 refute an obvious influence of ortet age on the growth performances and within-clone variations of the relevant offspring produced by microcuttings.

Table 3. Height (*H*), diameter at breast height (*D*), and volume (*V*) mean values ± standard error (SE) and coefficients of variation (CV) 6.5 years after the planting of clones 3, 7, and 8 produced by rooted cuttings or microcutings in trial 2, with the relevant number of observations (*N*) presented.

		H		*D*		*V*	
Clone (method)	*N*	Mean±SE (m)	CV (%)	Mean±SE (cm)	CV (%)	Mean±SE (dm^3)	CV (%)
3 (rooted cuttings)	55	16.2±0.5	23.9	16.5±0.6 ab	26.6	154.4±11.0 ab	53.1
3 (microcuttings)	44	15.6±0.4	18.5	15.3±0.5 ab	20.7	121.2±8.2 b	44.6
7 rooted cuttings	40	15.0±0.6	24.5	14.9±0.7 b	30.4	122.6±13.3 b	68.7
7 (microcuttings)	46	16.5±0.4	17.4	18.2±0.6 a	22.6	181.8±12.6 a	46.9
8 (rooted cuttings)	40	16.3±0.4	15.0	16.1±0.5 ab	20.1	139.4±10.2 b	46.4
8 (microcuttings)	32	15.8±0.3	13.0	15.9±0.5 ab	16.9	127.1±8.7 b	38.8

Note: Lowercase letters distinguish means that are significantly different at $P_o = 0.05$ for each growth trait (Student–Newman–Keuls test).

Trial 2: genotype and cuttings vs. microcuttings

Data means observed 6.5 years after planting are detailed in Table 3 for the six genotype × propagation method combinations compared with marked effects on *D* ($P = 0.0311$) and *V* ($P = 0.0005$). Interactions between these factors were significant for *H* ($P = 0.0178$), *D* ($P = 0.0167$), and, more particularly, for *V* ($P < 0.0001$). Overall, *H*, *D*, and *V* scores were slightly higher for the microcuttings than for the cuttings (16.0 vs. 15.9 m for *H*, 16.6 vs. 15.9 cm for *D*, and 145.6 vs. 140.5 dm^3 for *V*, respectively, for the three clones combined), but the differences in diameter and volume were significant for clone 7 only. Rooted cuttings induced a more pronounced within-sample variation than microcuttings for *H*, *D*, and *V*, as illustrated by their CV values. The same growth criteria did not differ statistically according the three genotypes compared, with rooted cuttings and microcuttings mixed.

Trial 3: genotype and microcuttings vs. meristem cultures

The mean scores of the six genotypes × propagation method combinations assessed 5 years after planting and reported in Table 4 had an influence only on *D* ($P = 0.0336$) and *V* ($P = 0.0185$). Noticeable block ($P < 0.0001$ for *H* and *D*, $P = 0.0140$ for *V*) and block × combination ($P = 0.0027$ for *H*, $P = 0.0008$ for *D*, and $P = 0.0042$ for *V*) effects were found. The mean and CV values of the measured traits varied substantially among the genotypes according to the method of propagation, especially for H, and consistently with the genotype × propagation method interaction found ($P = 0.0495$). Meristem culture derived plants of the three clones 1, 3, and 5 combined showed significantly larger diameters ($P = 0.0449$) and volumes ($P = 0.0073$) than the same material produced from microcuttings (17.4 vs. 16.0 cm for *D*, and 163.1 vs. 128.1 dm^3 for *V*), whereas height scores were similar (15.0 vs. 15.3 m).

Trial 4: mericlones

After 5 years of field growth, the two mericlonal lines from clone 3 showed comparable *H* (14.4–14.6 m; CV, 21.9%–22.1%), *D* (15.0–15.1 cm; CV, 33.9%–34.0%), and *V* (123.4–123.6 dm^3; CV, 77.4%–80.6%) mean values and within-sample variation. Block ($P < 0.0001$ for *H* and *V*, $P = 0.0005$ for D) and block × mericlone interactions ($P = 0.0093$ for *H*, $P = 0.0211$ for *D*, and $P = 0.0187$ for *V*) were the only significant effects pointed out by the analysis of variance.

Discussion

As far as we are aware, this is the first time that field growth performances of teak clones produced from genotypes of different ages by rooted cuttings, in vitro microcuttings, and SAM cultures are compared with sufficient data to allow for reliable statistical analyses. The high, constant precipitation regime at the planting site could have accounted for the good field survival and growth performances reported, which is consistent with Siswamartana and Wibowo's (2005) observations in Java and in contrast to places with less rain and a distinct dry season (Kaosa-ard 2000; Bekker et al. 2004; Nadgauda et al. 2005). Postplanting survival rates of more than 90% were also obtained for microcutting-issued teak plants in India (Nadgauda et al. 2005). However, the growth performances recorded remain superior to all field growth figures available to date for teak clones produced by rooted cuttings and established under similar rainfall conditions (Siswamartana and Wibowo 2005; Palanisamy et al. 2009). This suggests that high rainfall is not the only factor accounting for the better growth of our material. Site fertility, which has been assumed to have a strong influence on teak growth (Kadambi 1972; Tewari 1992), could be involved too.

Genetics also deserves special consideration. For example, clones 7 and 8 were observed to thrive not only in Taliwas, but also under a wide range of planting conditions, including much drier sites (Goh and Monteuuis 2012). Contrary to many arborescent species, the rooted cuttings and microcuttings of teak produced by efficient propagation techniques developed true to type, irrespective of the age of the ortet, and did not show maturation-induced C effects (Goh and Monteuuis 2005, 2012; Goh et al. 2007). Therefore, it is possible to produce clones from individuals sufficiently developed to express their phenotypic superiority, e.g., clones 13, 14, 15, 16, and 18 selected at a mature stage from outstanding teak stands that were growing in similar environmental conditions as Taliwas. This could explain the growth superiority of these materials compared with clones 9, 10, and 11, which were derived from much younger ortets and were insufficiently developed to be phenotypically selected on a reliable basis (Bonga 1982; Ahuja and Libby 1993; Monteuuis and Goh 1999). The differences in growth noticed between trials 1 and 2 for clones 3, 7, and 8 are assumed to be due to the variations in soil characteristics, depth especially, that are responsible for the block effects pointed out by the statistical analyses. Also, the narrow within-row spacing of 2 m between trees could have prematurely induced a strong competition for light and soil resources, increasing the between-tree differences of development, especially in height.

Although teak clones produced by rooted cuttings and microcuttings had similar growth performances, the particularities of these two clonal propagation techniques deserve further development. Until recently, the mass production of teak clones by rooted cuttings was considered as technically and economically applicable only to juvenile plants produced in vitro (Kjaer et al. 2000). The protocols developed in Sabah during the 1990s (Monteuuis 1995; Monteuuis et al. 1995) and their further successful transfer to various tropical countries have demonstrated their efficiency for mass clonally propagating by rooted cuttings a wide range of teak plus trees regardless of their age while preserving the integrity of the tree. The serial rooting propagation technique led more readily than serial bud grafting to physiological rejuvenation of mature selected genotypes (Husen and Pal 2003), and mean rooting rates of 70% can be obtained after two or three generations of "cascades" rooting (Monteuuis 1995; Monteuuis et al. 1995). This seems to be the minimal level of rooting responsiveness required for embarking on large-scale production of teak rooted cuttings in

Table 4. Height (*H*), diameter at breast height (*D*), and volume (*V*) mean values ± standard error (SE) and coefficients of variation (CV) 5 years after the planting of clones 1, 3, and 5 produced by microcutings or meristem culture in trial 3, with the relevant number of observations (*N*) presented.

		H		*D*		*V*	
Clone (method)	*N*	Mean±SE (m)	CV (%)	Mean±SE (cm)	CV (%)	Mean±SE (dm^3)	CV (%)
1 (microcuttings)	32	15.9±0.4	13.7	15.5±0.6 b	20.9	123.9±42.1 b	42.1
1 (meristem culture)	41	15.0±0.5	21.4	17.2±0.9 ab	34.7	162.1±17.4 ab	68.8
3 (microcuttings)	29	14.7±0.5	18.4	16.2±0.6 ab	20.5	129.6±12.1 ab	50.2
3 (meristem culture)	31	15.8±0.5	17.9	16.4±0.7 ab	22.6	143.3±13.0 ab	50.4
5 (microcuttings)	29	14.2±0.5	20.7	16.4±0.7 ab	24.7	131.1±12.1 ab	49.7
5 (meristem culture)	27	15.3±0.5	15.6	19.0±1.0 a	16.9	187.5±21.2 a	58.8

Note: Lowercase letters distinguish means that are significantly different at $P_o = 0.05$ for each growth trait (Student–Newman–Keuls test).

cost-efficient conditions. Two hundred and fifty thousand rooted cuttings per annum have been produced from mature selected teak genotypes in Tanzania by applying this procedure (H. Lemm, personal communication).

Notwithstanding a higher initial investment for setting up suitable tissue-culture facilities, the microcutting technique was found to be more economical in our conditions than the rooting of cuttings in a nursery for the production of more than 100 000 units per annum (Monteuuis 2000). This is due to several reasons. Mature selected genotypes can be physiologically rejuvenated more readily and more quickly under suitable in vitro protocols than under nursery conditions, resulting in a higher yield of clonal offspring produced in shorter delays and with less within-clone variation, as observed in this study. Microcuttings can be produced year-around, irrespective of climatic constraints such as marked dry seasons, and without requesting numerous stock plants that need to be properly and rigorously managed by experienced staff (Monteuuis et al. 1995; Monteuuis 2000). Another asset is the simplicity and cost efficiency of the protocol developed based on a unique elongation-multiplication in vitro culture medium (Monteuuis et al. 1998; Monteuuis 2000). The rooting is carried out with more than 90% success in cheaper and natural ex vitro conditions (Bonal and Monteuuis 1997), as also experienced with success in different places for juvenile (Yasoda et al. 2005) and mature (Daquinta et al. 2001; Castro et al. 2002; Tiwari et al. 2002) teak genotypes. This method has warranted the mass production of teak microcuttings for direct re-afforestation operations. This is in contrast with other forest tree species for which microcuttings are used in much lower quantities, due to cost constraints, for producing stock plants. These latter species are subsequently utilized for mass yielding rooted cuttings at a much cheaper cost in nursery conditions (Bonga and Von Aderkas 1992; Monteuuis 2009). Contrary to nursery plants, contamination-free in vitro microcuttings also meet the phytosanitation requirements to be exported to foreign countries for larger market prospects (Goh et al. 2007; Goh and Monteuuis 2012).

Another particularity of teak is the rather large size of its SAM compared with other tree species. This and its decussate leaf pattern facilitate its excision from the donor shoot, and skilled people can routinely inoculate 30 to 40 teak SAMs per hour onto proper in vitro culture media (Monteuuis et al. 1998). In addition to higher success rates and efficiency than nodal explants for initiating contamination-free cultures (70% vs. 20%–30% as respective mean success rates), especially in case of endogenous contaminants, SAMs are also more effective for achieving readily physiological rejuvenation from mature selected genotypes (Monteuuis 1989; Bonga and Von Aderkas 1992; Monteuuis and Goh 1999). This could account for the larger *D* and *V* scores noticed for the SAM-issued plants compared with the microcutting-derived ones. Although no noticeable difference could be detected between the only two mericlones assessed in this study, such investigations are useful for analyzing the possible influence of SAM initial location within the donor plant on the characteristics of its lineage in relation to ontogenetical ageing and the associated topophysis effects (Monteuuis 1989). In addition to being strongly justified from a theoretical point of view, these arguments plead for a more intensive use of SAMs for clonally propagating teak, considering that concrete applications of SAM culture to arborescent species remain to date still scarce (Bonga and Von Aderkas 1992).

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