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Ex Vitro Survival, Rooting and Initial Development of *in Vitro* Rooted *vs* Unrooted Microshoots From Juvenile and Mature *Tectona grandis* Genotypes

By D. BONAL and O. MONTEUUIS[1])

CIRAD-Forêt and ICSB Joint Project, P.O. Box 60793, 91017 Tawau, Sabah, Malaysia

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Summary

The influence of *in vitro* formed adventitious roots on acclimatization and initial *ex vitro* development of microshoots from juvenile and mature teak (*Tectona grandis*) genotypes was investigated. Overall, the *in vitro* rooted microshoots gave rise to higher survival and *ex vitro* rooting rates 7 weeks after transfer than those not rooted *in vitro*. The age difference resulted in higher mortality rates 7 and 15 weeks after transfer for the microshoots of mature origin. The number of roots produced *in vitro* was observed to have a strong influence on the number of roots formed *ex vitro* 7 weeks after transfer and on the height of the microshoots at the time of transfer, 7 weeks later and to a lesser extent after 15 weeks. Differences in height at transfer between microshoots from the two origins of plant material tended to disappear during the acclimatization process. Overall, more than 80% of the microshoots that were initially transplanted from the various categories tested were successfully acclimatized to *ex vitro* conditions. These results are discussed considering mainly the influence of the maturation process on the formation of *ex vitro* roots in *in vitro*-derived microshoots and emphasizing the role of some basic exogenous factors.

Key words: acclimatization, adventitious rooting, age, *ex-vitro* development, microshoots, *Tectona grandis*.

FDC: 165.44; 181.65; 176.1 *Tectona grandis*.

Résumé

L'influence de l'appareil racinaire de type adventif formé *in vitro* sur l'acclimatation et les premiers stades de développement *ex vitro* de microboutures de génotypes juvéniles et mature de teck (*Tectona grandis*) a été etudiée. Globalement, 7 semaines après leur transfert, les microboutures enracinées *in vitro* se distinguent par des taux de survie et d'enracinement *ex vitro* supérieurs à leurs homologues non enracinées *in vitro*. L'influence de l'âge se ressent au niveau des taux de mortalité plus élevés 7 et 15 semaines après le transfert pour les microboutures provenant du clone mature. Le nombre de racines formées *in vitro* influe sur le nombre de racines développées en conditions *ex vitro* 7 semaines après le transfert, ainsi que sur la hauteur des microboutures à la date du transfert, après 7 semaines, et dans une moindre mesure après 15 semaines. Les différences de hauteur mises en évidence entre les deux origines à la date du transfert tendent à s'estomper durant l'acclimatation. Plus de 80% de l'ensemble des microboutures transférées *ex vitro* ont pu être acclimatés avec succès. Ces résultats sont discutés en considérant principalement l'impact du phénomène de maturation sur la rhizogenèse *ex vitro* de microboutures issues d'*in vitro,* ainsi que l'influence de certains facteurs exogènes prépondérants.

Mots clés: acclimatation, âge, développement *ex-vitro*, enracinement adventif, microboutures, *Tectona grandis*.

Introduction

Tectona grandis, commonly known as teak, has gained a worldwide reputation as a high quality timber on account of the attractiveness and durability of its wood. This arborescent species occurs naturally although discontinuously in deciduous

[1]) Correspondence to: O. MONTEUUIS
Present address: CIRAD-Forêt, Campus de Baillarguet, BP 5035, F-34032 Montpellier Cedex 1, France
Phone: 33 4 6759 3787
Fax: 33 4 6759 3755/33
Email: monteuuis@cirad.fr

forests between 9° to 26° North latitude, and 73° to 104° East longitude, including central and southern India, Myanmar, northern Thailand and Laos. It has been subsequently introduced to many south-east Asia countries such as Indonesia, Sri-Lanka, Vietnam, West and East Malaysia, Solomon Islands and to some African countries like Ivory Coast, Nigeria and Togo. In 1990, the species was reported to cover about 25 millions ha, but the resources currently available are still far below the huge market demand. Planting stock is still produced from seeds despite certain handicaps such as quantitatively limited and late seed production, low germination rates, substantial variability in growth and wood quality among individuals within progenies (WHITE, 1991).

These limitations prompted us to explore whether mature selected genotypes could be clonally mass propagated true-to-type using nursery and tissue culture protocols (MONTEUUIS, 1995; MONTEUUIS et al., 1995b). For *in vitro* production, success is highly dependent upon the acclimatization process. In this respect, the formation of adventitious roots from a microshoot is the limiting factor (MCCLELLAND et al., 1990). The rooting process can occur *in vitro* or *ex vitro* depending on the species and the facilities available (DRIVER and SUTTLE, 1987).

The aim of this work was to compare *in vitro* rooted and unrooted microshoots of juvenile and mature teak genotypes with regard to their capacity to be successfully acclimatized to *ex vitro* conditions, to produce *ex vitro* roots and to resume early growth, considering the relative lack of accurate data available from the literature on this subject (MASCARENHAS et al., 1987; MASCARENHAS and MURALIDHARAN, 1993).

Material and Methods

Plant material origin and in vitro culture conditions

Microshoots from 2 different age origins of *Tectona grandis* were compared. The mature plant material was obtained from 1 cm to 2 cm long nodal explants collected from elongating shoots from the lower part of the crown of a 15 year-old *Tectona grandis* ortet growing outdoors at Luasong Forestry Centre, Sabah, East Malaysia. Juvenile explants were obtained from *in vitro* germinated seeds collected from the same ortet. Both origins systematically paired during all culture procedures were propagated through 1 cm to 2 cm long nodal microcuttings during 1 year alternating every 1.5 month subcultures on a basal multiplication and elongation culture medium (MONTEUUIS, 1995; BON and MONTEUUIS, 1996). This medium was solidified with 7gl^{-1} "high gel strength" Sigma agar after pH adjustment to 5.5 to 5.6, then autoclaved at 120 °C and 95 kPa for 20 min. All the cultures were maintained in 21 mm x 150 mm glass test tubes containing 10 ml of culture medium and covered with polypropylene caps under a 16-h photoperiod (50 to 60 µmol $m^{-2}s^{-1}$, "TLD 36W/84 Philips" fluorescent lamps) at 28/22 °C ± 2 °C light/dark. Transfer of the microshoots to *ex vitro* conditions occurred after 1.5 month of culture on the basal elongation medium where a large proportion of microshoots had already rooted spontaneously *in vitro*.

Ex vitro conditions

The 2 cm to 3 cm tall *in vitro* microshoots selected for the experiments (see hereunder for more information) were removed from the culture tubes and soaked for a few minutes in an aqueous fungicide solution – Thiram 80, 5g/L – before being inserted according to the experimental design into the rooting beds. Beds were filled with wet sand used as rooting substrate after it had been boiled to reduce disease risks.

Once set into the rooting bed, the microshoots were maintained under 50% shade with intermittent-mist water sprays provided by a mist system, the frequency of which was controlled by an "electronic leaf" system (HARTMANN et al., 1990) to avoid any desiccation damage. Aqueous fungicide solutions – mainly Thiram 80, 5 gl^{-1} – were sprayed on the microshoots weekly.

After 7 weeks the plantlets were carefully removed from the rooting substrate and potted individually in 10 cm x 15 cm black plastic bag containers filled with clayish local top soil, then maintained in the same *ex-vitro* conditions as described previously, except that the frequency of the misting was halved. The potted plants were kept 8 weeks under this regime, then removed from the mist-system to the field nursery.

Experimental designs, analysis criteria and statistical treatment of the data

Experiment 1

Survival capacity and rootability of *in vitro* rooted versus not rooted microshoots from juvenile and mature origins after transfer to *ex vitro* conditions were analyzed adopting a full factorial design involving the four combinations. Three contiguous randomized complete blocks were established, each including 4 elementary plots of 20 microshoots by combination. This experiment was first done on 9th of May 1995, then repeated using similar experimental conditions on the 10th of October 1995. This resulted in a total of 20 x 2 x 2 x 3 x 2 = 480 microshoots. Seven weeks after transfer into the rooting beds, the surviving microshoots were recorded according to their origin, distinguishing between the rooted and not rooted plantlets to establish the corresponding *ex vitro* rooting rate out of the microshoots still alive and the survival rates, based on the number of microshoots initially transferred to the rooting bed. Survival was recorded again for each plant material combination 8 weeks after potting (15 weeks after the date of transfer to *ex vitro* conditions), just before the potted plantlets were moved to the nursery.

The data were analyzed using the SPSS statistical package (SPSS inc., 1990). Null hypotheses were rejected for probability value $P \leq 0.05$. Tests for homogeneity of variance were performed using CROCHRAN's and BARTLETT's tests (SPSS Inc., 1990) which established the suitability of transforming the survival and rooting rates by arcsin. One-way analysis of variance, followed by the Least Significant Difference test "LSD" when the null hypothesis was rejected, was used to assess the influence of the different categories of plant material on survival and *ex-vitro* rooting and then to compare the corresponding means (SPSS Inc., 1990). The effects of the experimental factors on the same traits were assessed carrying out an analysis of variance, ANOVA (SPSS Inc., 1990), of the following model:

$$Y_{ijkl} = \mu + R_i + O_j + D_k + B_l + (RO)_{ij} + (RD)_{ik} + (RB)_{il} + (OD)_{jk} + (OB)_{jl} + (DB)_{kl} + \varepsilon_{ijkl}$$

where:

Y_{ijkl}: value of the plot submitted to the i^{th} level of factor *in vitro* "rooting", j^{th} level of factor "origin", k^{th} level of factor "date of experiment" and l^{th} level of factor "block";

µ: "grand mean" of the whole experiment;

R_i: effect of the factor *in vitro* "rooting", $1 \leq i \leq 2$;

O_j: effect of the factor "origin" of the microshoots, $1 \leq j \leq 2$;

D_k: effect of the factor "date", $1 \leq k \leq 2$;

B_l: effect of the factor "block", $1 \leq l \leq 3$;

$(RO)_{ij}$: effect of the interaction between the factors "rooting" and "origin";

$(RD)_{ik}$: effect of the interaction between the factors "rooting" and "date";

$(RB)_{il}$: effect of the interaction between the factors "rooting" and "block";

$(OD)_{jk}$: effect of the interaction between the factors "origin" and "date";

$(OB)_{jl}$: effect of the interaction between the factors "origin" and "blocks";

$(DB)_{kl}$: effect of the interaction between the factors "date" and "blocks";

ε_{ijkl}: random error.

Experiment 2

The influence of the number of roots formed *in vitro* on survival, root production and growth of the microshoots after transfer to *ex vitro* conditions was examined by selecting for each origin 10 *in vitro* microshoots for each of the following classes: no root, 1 root, 2 roots, 3 roots (restricted to 5 microshoots from the mature origin), and 4 roots or more (from the juvenile origin only as none was available from the mature origin). These microshoots were transferred to the rooting bed in a completely randomized single-microcutting plot design, with a regrouping of the juvenile and the mature origins together and individually identified with a label. In addition to the survival rates defined previously, records were made for each labelled plantlet in order to determine the following criteria: (*i*) the height (H0) of the plantlet just after setting in the rooting bed; (*ii*) the height at 7 weeks (H7) and (*iii*) the number of roots (NRex) developed after 7 weeks in the rooting bed and just prior to potting; (*iv*) the height H8 just after potting; (*v*) the height H15 8 weeks after potting (15 weeks from the date of transfer to *ex vitro*) and before dispatch to the nursery; (*vi*) the height increment HI1 7 weeks after transfer to *ex-vitro* conditions (HI1 = H7 – H0); (*vii*) the height increment HI2 8 weeks after potting (HI2 = H15 – H8) and (*viii*) the difference DNR between the number of roots *in vitro* just before transfer and the number of roots recorded *ex vitro* 7 weeks later.

This second experiment was conducted under the same experimental conditions and on the same dates as Experiment 1. A total of 170 microshoots, $10 \times 5 \times 2 = 100$ for the juvenile genotypes and $(10 \times 3 \times 2) + (5 \times 2) = 70$ for the mature clone were observed. The influence of the different categories of plant material on the various traits observed and the relevant mean comparisons were analyzed applying the same statistical procedures as described for Experiment 1, with data transformed by $\log_{10}$ to fulfil homogeneity of variance requirements when needed. Except for the survival rates for which the Chi-square test was used (SPSS Inc., 1990), the effect of the different experimental factors on the various traits observed was assessed through an analysis of variance of the following model:

$$Y_{ijk} = \mu + N_i + O_j + D_k + (NO)_{ij} + (ND)_{ik} + (OD)_{jk} + \varepsilon_{ijk}.$$

where:

Y_{ijk}: value of the plot corresponding to the i^{th} level of factor "number of *in vitro* roots", the j^{th} level of factor "origin" and the k^{th} level of factor "date of experiment";

μ: grand mean;

N_i: effect of the factor "number of *in vitro* roots" of the microshoots, $1 \le i \le 4$;

O_j: effect of the factor "origin", $1 \le j \le 2$;

D_k: effect of the factor "date", $1 \le k \le 2$;

$(NO)_{ij}$: effect of the interaction between the factors "number of *in vitro* roots" and "origin";

$(ND)_{ik}$: effect of the interaction between the factors "number of *in vitro* roots" and "date";

$(OD)_{jk}$: effect of the interaction between the factors "origin" and "date";

ε_{ijk}: random error.

Due to imbalance in the data between the 2 origins of plant material, the microshoots with 4 roots or more developed *in vitro* were excluded from the analysis of variance.

Results

Experiment 1

The 4 categories of microshoots were found to noticeably influence the *ex vitro* rooting rate ($P = 0.001$) and survival scores at 7 weeks ($P = 0.009$) and 15 weeks ($P = 0.010$) after transfer from *in vitro* as illustrated in *figure 1*. The analysis of variance (*Table 1*) established that the *in vitro* rooting status of the microshoots prior to acclimatization had an influence on survival after 7 weeks, to the advantage of the microshoots rooted *in vitro* compared to the not rooted ones (93.8% vs 85.46%, respectively). However, this effect was no longer significant 15 weeks after transfer. *In vitro* rooted microshoots also gave rise to higher *ex vitro* rooting rates than those transferred from *in vitro* without roots (98.4% vs 83.7% respectively). Differences in age origin of the microshoots resulted in significant differences in survival 7 weeks and 15 weeks after transfer to *ex vitro* conditions (*Table 1*), with higher scores for the juvenile plant material compared to the mature clone (95.4% vs 83.7%, and 94.2% vs 81.3%, respectively). A significant interaction between the rooting status *in vitro* and the age origin was found only for the *ex vitro* rooting rates (*Table 1*), with the greatest difference observed in that respect between the *in vitro* rooted versus unrooted microshoots of the mature origin, as shown in *figure 1*.

Table 1. – Results from the analyses of variance (significance levels, F-test) performed for the various traits investigated in relation to the different experimental factors assessed in Experiment 1 (see text for more information).

Source of variation	df	Traits		
		Surv.7	Surv.15	Root.
In vitro rooting status (R)	1	0.036*	0.071	0.001***
Origin (O)	1	0.005**	0.010**	0.664
Dates (D)	1	0.253	0.810	0.741
Blocks (B)	2	0.910	0.933	0.069
R X O	1	0.641	0.419	0.024*
R x D	1	0.063	0.100	0.563
R x B	2	0.251	0.347	0.762
O x D	1	0.229	0.364	0.125
O x B	2	0.213	0.226	0.059
D x B	2	0.347	0.434	0.757

Surv. 7 and Surv. 15: survival rates of the microshoots respectively 7 weeks and 15 weeks after transfer; Root.: rooting rates 7 weeks after transfer. The data were transformed by arcsin for homogeneity of variance requirements. Asterisks indicate a significant effect of the experimental factors on the trait considered at *) $P \le 0.05$, **) $P \le 0.01$ and ***) $P \le 0.001$ levels of significance.

Experiment 2

The different categories of microshoots assessed influenced the height of the plant material (*i*) at the time of transfer to *ex vitro* conditions (HO, $P < 0.001$), (*ii*) after a period of 7 weeks (H7, $P < 0.001$), and (*ii*) after 15 weeks (H15, $P = 0.004$). Microshoots without any roots *in vitro*, or those with only one root from the juvenile origin were significantly shorter than the other categories at the time of transfer, as illustrated in *figure 2*. This trend was still present 7 weeks after transfer, whereas the average height of the juvenile microshoots not rooted *in vitro* was comparable to those of the other categories after 15 weeks (*Figure 2*). Height increment following transfer was not significantly influenced by the different microshoot categories, unlike the number of *ex vitro* roots ($P = 0.002$). For this latter criterion, *figure 3* shows that the highest scores were obtained for the juvenile microshoots which had formed 3 and 4 roots *in vitro*.

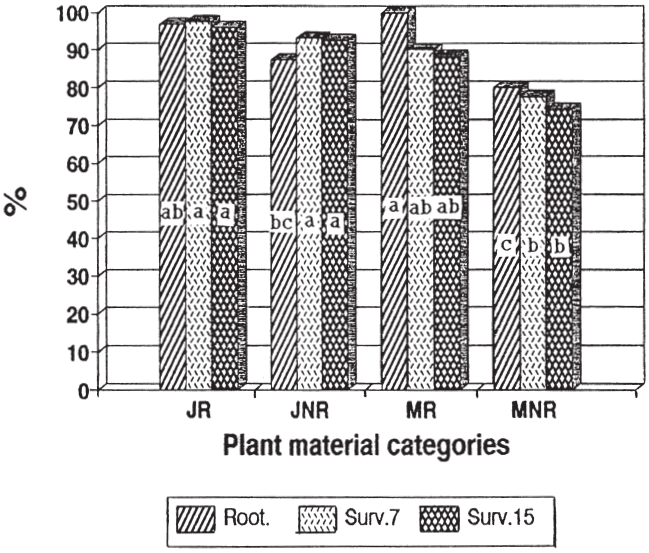

Figure 1. – Mean ex-vitro rooting rates (Root.) and survival rates 7 weeks (Surv. 7) and 15 weeks (Surv. 15) after transfer to ex-vitro conditions of the 4 categories of microshoots assessed in Experiment 1, i.e. juvenile rooted in vitro (JR), juvenile not rooted in vitro (JNR), mature rooted in vitro (MR) and mature not rooted in vitro (MNR). For each of the 3 criteria observed, letters distinguish means which are significantly different at the 5% level (LSD test).

Results from the analysis of variance considering each experimental factor investigated independently of the others are given in *table 2*. The height of the microshoots was strongly influenced by the number of roots formed *in vitro*, at the time of transfer, 7 weeks later and to a lesser although still significant extent after 15 weeks, which was consistent with the significance levels observed for HI1 and HI2. Overall, the microshoots from the mature origin were taller than the juvenile ones at the time of transfer (1.76 cm vs 1.42 cm). This difference in height between the 2 origins was still present but less obvious after 7 weeks (3.38 cm vs 3.20 cm and disappeared after 15 weeks. The date of transfer noticeably affected the height of the microshoots *ex vitro* recorded after 7 weeks, as well as the height increment measured at that time. The number of *ex vitro* roots was markedly influenced by the number of roots formed previously *in vitro*, with a significant date effect pointed out also for the difference (DNR) between the number of roots formed *in vitro* and those produced *ex vitro* (*Table 2*). Overall, higher scores were obtained for the microshoots transferred in May (data not shown).

Interactions between the age origin of the microshoots and the number of roots produced *in vitro*, which was most obvious for the microshoots with one root *in vitro* as shown in *figure 2*, were found significant only for height after 7 weeks and 15

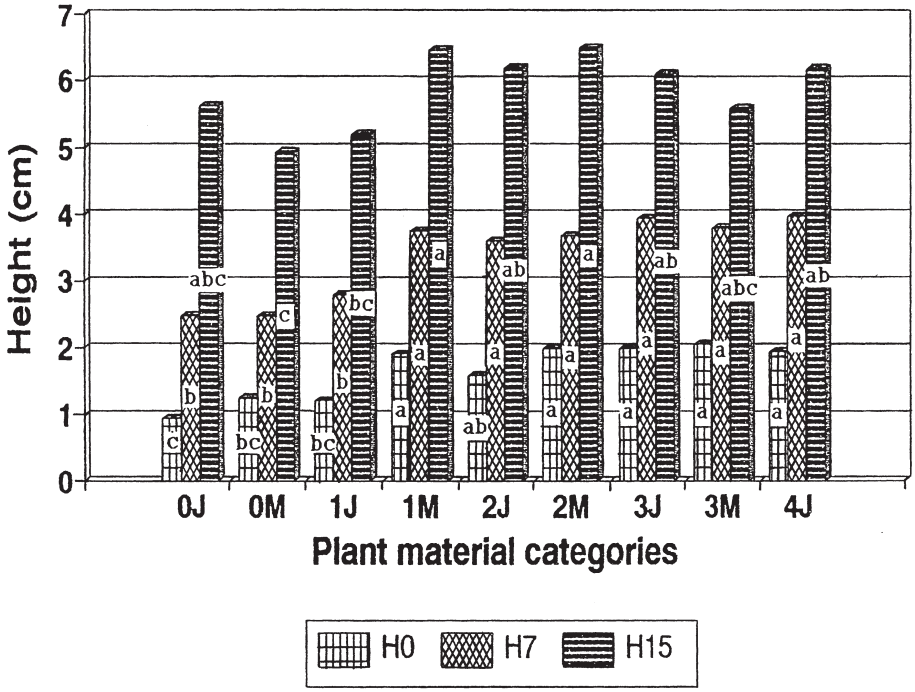

Figure 2. – Mean height measurements *i*) just after transfer HO, *ii*) 7 weeks after transfer H7 and *iii*) 15 weeks after transfer H15 (8 weeks after potting) to ex-vitro conditions of the different categories of microshoots assessed in Experiment 2, i.e. without any root in vitro from juvenile (0J) and mature (0M) origins, with 1 root in vitro from juvenile (1J) and mature (1M) origins, with 2 roots in vitro from juvenile (2J) and mature (2M) origins, with 3 roots in vitro from juvenile (3J) and mature (3 M) origins and with 4 roots in vitro from juvenile (4J) origin. For each of the 3 heights considered, letters distinguish means which are significantly different at the 5% level (LSD test).

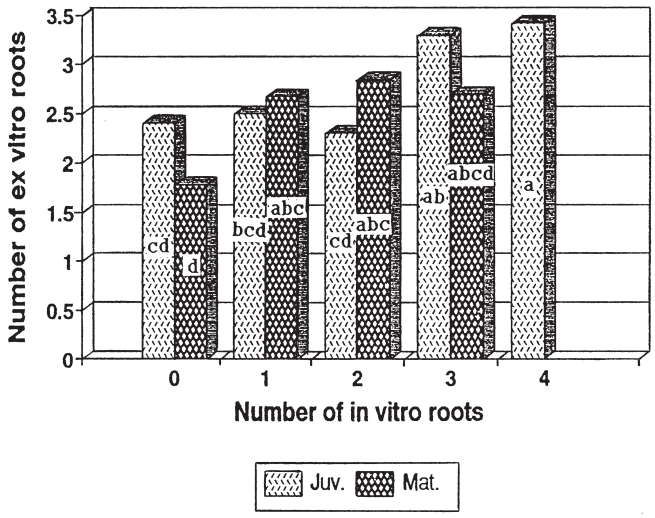

Figure 3. – Average number of ex-vitro roots formed by the microshoots 7 weeks after transfer, in relation to their age origin and the number of in vitro roots they had at the time of transfer in Experiment 2. Letters distinguish means which are significantly different at the 5% level (LSD test).

weeks. Corresponding height records, as well as height increment measurements after 7 weeks (HI1), were observed to be much higher overall for the microshoots of the juvenile origin transferred in May compared to the other origin X date combinations, as reflected by the significant relevant interactions (*Table 2*).

Discussion

The practical prospects of mass micropropagation are largely dependent on the success of the acclimatization process and the capability of the acclimatized plantlets to resume growth. In that respect and based on previous observations, it seems founded to devote special attention to the *in vitro* rooting status of the microshoots prior to acclimatization, which is liable to vary according to the age of the plant material (HACKETT, 1985, 1988) and to the genotype (HAISSIG and RIEMENSCHNEIDER, 1988). Genetically closely related mature and juvenile plant material origins were utilized in order to minimize the genetic effects.

Adventitious roots formed *in vitro* have been reported for different species to display particular morphological and anatomical features induced by the physical characteristics of the gelled culture medium (MOHAMMED and VIDAVER, 1988; McCLELLAND and SMITH, 1988, McCLELLAND et al., 1990). Such roots may stimulate growth *in vitro* as established by the measurements made at the time of transfer but in the same way as noticed for other tree species (McCLELLAND et al., 1990) *in vitro* formed roots did not seem to be adapted to more natural conditions. Indeed, most of them were observed to disappear after transfer to the sand used as rooting substrate, to be replaced by more slender, branched *ex vitro* produced roots with whitish tips. These roots were assumed to be more functional than those formed *in vitro* (McCLELLAND et al., 1990). When originating from root tissues initiated *in vitro*, the new *ex vitro* roots must be considered from the anatomical standpoint, more as a result of a root regeneration process than as a *de novo* formation of adventitious roots from shoot tissues depending upon the capability of the latter to dedifferentiate (HARTMANN et al., 1990). This basic distinction of the origin of the roots developed *ex vitro* could account for the differences observed after transplanting between the *in vitro* rooted and unrooted microshoots as regards the *ex-vitro* rooting rates especially when referring to the mature origin. Adventitious rooting has been reported to be more difficult to achieve from mature tissues than from juvenile ones (HACKETT, 1988). *In vitro* conditions have also been proven for different woody species to be more suitable for rooting shoots of mature origin than *ex vitro* conditions (FRANCLET, 1983; HACKETT, 1988; McCOWN, 1988). This could be due to the possibility to rejuvenate the mature tissues through successive transfers onto appropriate *in vitro* media, thereby inducing a progressive enhancement of their potential for adventitious rooting (FRANCLET, 1983; HACKETT, 1985, 1988). At the time of transplant however, some of the microshoots of the mature origin had not rooted spontaneously *in vitro*, possibly because they were not sufficiently rejuvenated to display the same capability for *ex vitro* adventitious rooting as their homologs from the juvenile origin. This lesser ability for adventitious root formation after transfer to *ex vitro* conditions likely induced higher mortality rates as compared to the juvenile origin. Thus, for this kind of plant material, it seems advisable to root the microshoots *in vitro*, prior to their transfer to *ex vitro* conditions. Another option which may be worth testing is to dip the base of the microshoots not rooted *in vitro* into a rooting powder prior to setting in the rooting beds, as recommended for *Sequoia sempervirens* (POISSONNIER et al., 1980). Depending on the species, treatment with exogenous auxin can stimulate the potential for *ex vitro* rooting, while avoiding an additional transfer of the microshoots onto a special *in vitro* rooting medium. Practically, *ex vitro* rooting of tissue-cultured microshoots offers basic advantages and results in a substantial gain of money (McCOWN, 1988).

Table 2. – Results from the analyse of variance (significance levels, F-test) performed for the various traits investigated (see text for definition) in relation to the different experimental factors assessed in Experiment 2.

Source of variation	df	Traits						
		H0	H7	H15	HI1	HI2	NRex	DNR
No of roots in vitro	3	<0.001***	<0.001***	0.026*	0.003**	0.111	0.001***	0.070
Origin	1	<0.001***	0.030*	0.537	0.158	0.177	0.192	0.373
Dates	1	0.442	<0.001***	0.391	<0.001***	0.255	<0.001***	0.002**
No of roots in vitro X Origin	3	0.126	0.048*	0.029*	0.138	0.363	0.236	0.248
No of roots in vitro X Dates	3	0.079	0.332	0.282	0.591	0.322	0.008	0.092
Origin X Dates	1	0.635	0.007**	0.028*	0.003*	0.474	0.453	0.380

H0, H7, NRex and DNR data were transformed by $\log_{10}$ for homogeneity of variance requirements.
Asterisks indicate a significant effect of the experimental factors on the trait considered at *) $P \leq 0.05$, **) $P \leq 0.01$ and ***) $P \leq 0.001$ levels of significance.

As far as the influence of exogenous factors on *ex vitro* adventitious rooting is concerned (RAUTER, 1983), special consideration has to be devoted to the mist system (MCCOWN, 1988; MOHAMMED and VIDAVER, 1988; DEBERGH, 1991). Use of mist enabled us to maintain the survival of the different categories of microshoots, especially those not rooted *in vitro*, for the time needed to develop *ex vitro* roots and to get them finally acclimatized with overall success rates of more than 80%. This score is far superior to the data available so far from the literature in the same field on the same species based on the use of a specific *in vitro* rooting medium (MASCARENHAS et al., 1987). The protocol developed in our conditions has been useful for successfully acclimatizing 50.000 microshoots of teak to date, mainly from mature genotypes. Another important factor with regard to adventitious rooting is the rooting substrate (RAUTER, 1983; HARTMANN et al., 1990). Sand was used as it was locally more easily available than other components which might have been more appropriate to increase the rooting rates and consequently the acclimatization success (MOHAMMED and VIDAVER, 1988; HARTMANN et al., 1990). Light has been also observed to influence adventitious root formation differently according to the species (THOMPSON, 1992). In our case, longer photoperiod in May could have stimulated the formation of *ex vitro* roots and consequentially the early growth of the microshoots compared to those transferred in October. Conversely, under the same experimental conditions, the potential for adventitious root formation of *Acacia mangium* cuttings was observed to be weaker in May than in October (MONTEUUIS et al., 1995a). As a matter of fact, although slight under these latitudes, differences in photoperiod during the year have been noticed to have an effect on plant physiology as reflected by changes in vegetative characteristics, such as shoot growth variation and leaf fall observed locally for several tree species.

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