

A comparison of the field behaviour of ramets derived from a mature tree of *Acacia mangium* by tissue culture and the seedling progeny of the ortet

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Summary

The field behaviour of ramets of *Acacia mangium* produced *in vitro* from a 4-y-old selected 'plus tree' was compared to the field behaviour of the half-sib seedling progeny of the ortet. Assessments of mortality, frequency of multiple stems, susceptibility to wind breakage, total height, and diameter at breast height, were made over a period of 36 months from the planting date. Only mortality at 36 months differed significantly between the ramets and the seedlings, the rates being 23% and 0% respectively.

Keywords: vegetative propagation; tissue culture; plant development; growth; seedlings; *Acacia mangium*

Introduction

Acacia mangium Willd. is a tropical tree belonging to the family Leguminosae, sub-family Mimosoideae. Originating from Papua New Guinea (PNG), eastern provinces of Indonesia and north-eastern Queensland in Australia (Pinyopusarerk *et al.* 1993), this species is of increasing interest as an exotic for reforestation in many tropical countries. This interest is particularly obvious in South-East Asia, where the tree was first introduced in Sabah in 1966. The striking expansion of *A. mangium* plantations within a few years, mainly for pulpwood production, is due to the species' impressive growth under humid tropical conditions, especially on acid and degraded soils, which *A. mangium* plantations may improve through natural fixation of nitrogen.

Vegetative propagation, and more particularly cloning, has been proposed as a promising option for enhancing the yield and quality of *A. mangium* plantations (Haines and Griffin 1992; Walker and Haines 1998). Consequently, the ability of the species to produce adventitious roots in the nursery as well as in tissue culture has been investigated (Darus 1993; Poupard *et al.* 1994; Monteuis *et al.* 1995; Bon *et al.* 1998; Monteuis and Bon 2000). As far as we are aware, however, any practical benefit resulting from the use of clones derived from cuttings rather than seedlings for establishing *A. mangium* in industrial plantations has yet to be demonstrated.

The aim of this paper is to provide preliminary information on the field behaviour of ramets of a selected mature *A. mangium* compared to the field behaviour of its half-sib seedling progeny.

Materials and methods

Planting material

The clone was produced *in vitro* according to the procedure described in Monteuis and Bon (2000). It originated from the biggest 4-y-old *A. mangium* tree within a provenance/progeny trial established at Luasong Forestry Centre, Sabah, East Malaysia. This 'plus tree', from a PNG provenance, had commenced flowering and could therefore be considered to be physiologically mature (Hackett 1985). After acclimatisation under mist-system conditions, the rooted microshoots were individually potted in 10 cm × 15 cm black plastic bag containers filled with clayish local topsoil. They were raised for 3 months in the nursery to reach a size suitable for planting in the field. Only plants exhibiting an orthotropic growth pattern were kept; plagiotropic ones (about 30% of all cuttings) were discarded.

The seedling plant material consisted of open-pollinated seedlings from the *A. mangium* ortet grown from seeds collected after selective thinning of the provenance/progeny trial. Only one tree, the biggest of the five individuals initially planted in each progeny plot, had been kept. These half-sib seedlings were raised at the same time and under the same nursery conditions as the ramets, until they reached the same average size, 40 cm high.

Experimental design, analysis criteria and statistical treatment of the data

The two types of planting material were planted in a randomised complete block design, each origin being represented by 3 plots of 10 individuals. This resulted in a total of $10 \times 3 \times 2 = 60$ individuals. The 3×2 contiguous randomised blocks were surrounded by a two-row buffer planted with *A. mangium* seedlings of the same PNG provenance and of the same height as the experimental material. A standard spacing of 3 m × 3 m was used throughout.

The two origins were compared with regard to the following traits:

- (1) mortality;
- (2) multiple (more than one main stem) stem formation;
- (3) susceptibility to breakage by wind;
- (4) height from the bottom to the top of the tree;
- (5) diameter at breast height (DBH) (130 cm; the diameter corresponding to the sum of the sectional areas was used for trees with multiple stems).

Data were recorded for each tree at five different dates: T0 = just after planting; T1 = T0 + 8 months; T2 = T0 + 16 months; T3 = T0 + 26 months; T4 = T0 + 36 months.

Results are expressed as means based on 30 observations, or slightly fewer depending on mortality, for each of the two origins.

The data were analysed independently for each of the five dates. The χ^2 -Pearson's test (proportions) was used for traits 1, 2 and 3, whereas an analysis of variance (*F* test, SAS GLM procedure, SAS Institute Inc. 1996) was used for traits 4 and 5 (after data transformation when necessary according to Bartlett's test for homogeneity of variance (Sokal and Rohlf 1995)).

A probability level of $P \leq 0.05$ was considered significant for all the statistical analyses.

Results and discussion

This study was carried out over a period of 36 months. This can be considered as half the rotation length for highly productive *A. mangium* plantations of suitable origin intensively managed with a view to the production of pulpwood (Monteuuis and Nasi 1992). Of the five traits investigated at five different dates, only mortality at 36 months after planting differed significantly ($0.001 < P < 0.01$) according to the origin of the planting material. The mortality of the ramets increased gradually, and finally reached 23% (7/30) after 36 months, while no loss at all was observed in the seedling progeny within the same period (Table 1).

The other criteria were not significantly influenced by the two different sources of planting material during the period of investigation.

The nature of the root systems could account for the marked difference in mortality. The ramets in the clone were derived from microcuttings which, *in vitro*, produced a few frail and easily-damaged adventitious roots (Monteuuis and Bon 2000). In contrast, the seedlings developed more vigorous tap roots. Several studies have already established that vegetative propagation of *A. mangium* either by cuttings in nursery conditions (Poupard *et al.* 1994; Monteuuis *et al.* 1995) or microcuttings in *in vitro* culture (Bon *et al.* 1998; Monteuuis and Bon 2000) can be seriously handicapped by the poor quality of the newly-formed root system. This is particularly true for clones of mature genotypes: the capacity of *A. mangium* for true-to-type cloning (Monteuuis 1995), and especially for adventitious rooting (Poupard *et al.* 1994; Monteuuis *et al.* 1995; Monteuuis and Bon 2000), seems to be adversely affected early in life by the maturation process.

The fact that microshoots from a mature ramet may rejuvenate sufficiently from a physiological standpoint to be rooted *in vitro* does not mean that rejuvenation is complete (Hackett 1985; Pierik 1990). For instance, just after acclimatisation in the nursery, some of the rooted ramets showed plagiotropic growth that could be interpreted as symptoms of topophysis (Olesen 1978) resulting from the persistence of a certain degree of maturation within their tissues. In addition, the bark at the bottom of ramets was brightly colored, similar to that seen in the crown of mature plants, whereas the bark of the seedlings was dark brown, as would be expected in seedlings of PNG origin. Such characteristics are due to cyclophysis (Olesen 1978) or ontogenetical ageing (Fortanier and Jonkers 1976), demonstrating that tissue from the mature ortet had been incompletely rejuvenated. Effects of persisting maturation could also account for the within-sample or intra-clonal variability (Hackett 1983) of the ramets for height and DBH as reflected in the standard deviations in Table 1, unexpectedly similar to those of the seedlings, if not slightly greater.

Table 1. Mean values of the five traits assessed in ramets (R) and seedlings (S). Letters distinguish means which are significantly different at $P < 0.01$ ($\chi^2 = 7.92$).

Date	Mortality		Frequency of multiple stems		Frequency of wind breakage		Height* (cm)		DBH* (cm)	
	S	R	S	R	S	R	S	R	S	R
T0	0/30	0/30	0/30	0/30	0/30	0/30	36 ± 2	35 ± 3		
T1	0/30	2/30	1/30	1/28	0/30	0/28	340 ± 23	343 ± 28	—	—
T2	0/30	3/30	3/30	1/27	0/30	0/27	820 ± 37	792 ± 48	8.1 ± 0.7	7.4 ± 0.7
T3	0/30	3/30	3/30	1/27	0/30	2/27	1357 ± 37	1272 ± 61	12.1 ± 0.7	11.6 ± 0.9
T4	0/30 ^a	7/30 ^b	3/30	1/23	5/30	3/23	1550 ± 81	1609 ± 98	14.4 ± 0.9	13.7 ± 1.2

* Results expressed as means ± standard error at $P = 0.05$. Individual data were submitted to an analysis of variance of the following model: $Y_{ij} = \mu + O_i + B_j + (OB)_{ij} + \epsilon_{ij}$, with Y_{ij} = value of the plot submitted to the i th level of factor 'origin' and j th level of factor 'block'; μ = grand mean of the whole experiment; O_i = effect of the factor 'origin', $1 \leq i \leq 2$; B_j = effect of the factor 'block', $1 \leq j \leq 3$; $(OB)_{ij}$ = effect of the interaction between the factors 'origin' and 'block'; ϵ_{ij} = random error.

Another factor to consider is the genetic neighbourhood of the tree from which the seeds were collected. Seed stand design and more precisely the respective location of potential male parents, with their particular genetic origin and characteristics, will influence the genetic make-up of the progeny and hence the results of a study such as this. For instance, the fact that ultimately only the best tree of each progeny plot was retained improved the average genetic quality of the parents and reduced the risk of inbreeding depression, enhancing field performance of resulting seedlings.

Notwithstanding its limited scale, this study suggests that the likely practical benefit from the use of clonal propagules — especially those derived from mature trees — to establish *A. mangium* plantations should be critically assessed. Greater gains can be obtained at less cost through wisely-established seed production areas (Monteuuis and Nasi 1992).

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