resistance to environmental stresses have been reported by KNOWLES and MITTON (1980), KNOWLES and GRANT (1980), LEDIG *et al.* (1983), LARSEN (1986).

The vigorous growth, of the few imported hybrid trees, under contrasting environmental conditions in Israel points to success of afforestations with: (i) local artificially developed hybrids between plus trees of Israeli *P. halepensis* X *P. brutia* and/or (ii) selection of plus trees among the identified hybrid trees and their vegetative propagation for the establishment of seed orchards.

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In Vivo Grafting and *In Vitro* Micrografting of *Acacia mangium*: Impact of Ortet Age

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Abstract

The possibilities of vegetatively propagating juvenile – 6-month-old – and mature – 3 to 5 year-old – *Acacia mangium* ortets by grafting were investigated using *in vivo* and *in vitro* techniques. The average success rates obtained for *in vivo* top-cleft grafting were 49% for scions coming from juvenile plant material and 0% when collected from mature ortets. *In vitro* micrografted apices gave rise to 52% and 46% of successfully established micrografts for the juvenile and the mature plant material respectively. No significant difference between juvenile and mature origins in terms of grafting success was observed for *in vitro* micrografting of shoot apices. However, the ones coming from the juvenile ortets elongated more readily than those from the mature origin which were more prone to rest. Overall, the *in vitro* micrografting technique used appeared to be an helpful tool for vegetative non-destructive propagation of mature selected *Acacia mangium* ortets, apparently recalcitrant to more conventional *in vivo* grafting techniques.

These results are discussed in terms of scion size and the related potential for grafting in relation to the age of the ortet.

Key words: Acacia mangium, age, grafting, *in vitro*, micrografting, ortet, shoot apex, vegetative propagation.

FDC: 165.442; 176.1 *Acacia mangium*.

Introduction

Grafting has been extensively used for centuries for asexually propagating tree species, mainly for fruit production. This vegetative propagation technique is still broadly utilized in

horticulture (HARTMANN et al., 1990) but also for mass clonal propagation of rubber trees. In forestry, grafting has been mainly used for seed orchard establishment or as a source of vegetative propagules for clonal forestry (ZOBEL and TALBERT, 1984).

Micrografting has been developed more recently (BURGER, 1985; JONARD, 1986) and consists of grafting in aseptic conditions of a miniaturized scion onto an *in vitro* grown rootstock. The resulting *in vitro* micrograft and the plant material deriving from it can be further cultivated in tissue culture conditions, or acclimatized to outdoors. In addition to the benefits of traditional grafting, micrografting tiny shoot tips can be an efficient means of regenerating plant material free of endogenous contaminants (JONARD, 1986; HARTMANN et al., 1990) and with enhanced potential for true-to-type cloning from mature plants (FRANCLET, 1983). The possibility to micrograft less differentiated shoot tip tissues may help also in reducing compatibility problems between scion and stock (LACHAUD, 1975; JONARD, 1986).

The benefits of applying grafting and micrografting to *Acacia mangium* are obvious, considering the need to improve the genetic quality of the planting stock of this attractive fast growing species with increasing crop potential under humid tropics, especially in South East Asia. So far, indeed, air layering or "marcottage" (HARTMANN et al., 1990), although not easy to perform and in spite of rather moderate success, has been the most widely used non destructive method to vegetatively multiplying mature *Acacia mangium* trees recalcitrant to propagation by cuttings (POUPARD et al., 1994).

The capacity for grafting and micrografting of juvenile and mature *Acacia mangium* trees was therefore investigated and the results are reported in this paper.

Material and Methods

Scion source

Mature plant material scions were collected from the basal part of the crown of *Acacia mangium* trees, provenance Papua New Guinea, growing outdoors in Sabah (East Malaysia), 3-year-old since seed germination when the first grafting experiments started, and which just entered the flowering stage.

Juvenile plant material scions originated from 6 to 8 month-old seedlings, provenance Papua New Guinea, container-cultivated in the nursery, close to the mature ortets.

Several sample collections were made at different dates simultaneously for the two age classes which were systematically paired during all the grafting and micrografting procedures.

Grafting technique

Preliminary experiments (data not reported) established the superiority of the "top-clef" grafting technique (HARTMANN et al., 1990) over various other types of grafting for *Acacia mangium*, and this was therefore selected. To ensure a proper matching of stem size between scions and rootstocks, mature plant material scions were collected from the basal part of 10 cm to 20 cm long epicormic shoots arising from low branches of the trees, whilst juvenile scions were taken at about two thirds of the total height of the seedlings. Regardless of the age class, scions consisted of 2 node stem portions, 5 cm to 7 cm in length, with about two thirds of the surface of the phyllodes removed in order to lower evapotranspiration and to reduce water stress risks. The longer basal internode, trimmed to form a "V" 15 mm to 25 mm in length, was then inserted into the vertical slit made in the central part of the one third decapitated stem, about 3 mm in diameter, of the rootstock, a 6 to 8 month-old seedling of the same characteristics as the ortets used as source of juvenile plant material scions. Parafilm tape was used to tie the scion to the stock, and the union was covered with grafting wax, following the recommendations for top-cleft grafting (HARTMANN et al., 1990). The grafted stocks were then placed under 50% shade with intermittent-mist water sprays provided by a mist system (POUPARD et al., 1994) during 3 to 4 weeks to avoid any desiccation damage until the union was successfully established.

Micrografting technique

The *in vitro* rootstocks were obtained from *Acacia mangium* seeds, provenance Papua New-Guinea, first soaked for 5 seconds to 10 seconds in boiling water, then surface-sterilized by immersion for 5 min in 70% ethanol and then in 1‰ $HgCl_2$ aqueous solution. After 3 abundant rinses in sterilized ultra-pure water, the seeds were placed individually in aseptic conditions on 20 mm x 30 mm "Sorbarod" cellulosic plugs in 21 mm x 150 mm glass test tubes covered with polypropylene caps. The plugs had been previously saturated with 5 ml of a liquid medium consisting of half-strength MURASHIGE and SKOOG (1962) macro and micronutrients, with 20 gl^{-1} sucrose and with pH adjusted to 5.5 to 5.6 prior to autoclaving at 120 °C and 95 kPa for 20 min. Cultures were maintained under a 16-h photoperiod (50 to 60 µmol m^{-2} s^{-1}, "TLD 36W/84 Philips" fluorescent lamps) at 28/22 ± 2 °C light/dark. In these conditions 60% to 80% of the seeds germinated to develop within 2 to 3 months into young seedlings with elongating epicotyl suitable for grafting.

The scions used for micrografting originated from shoot apical portions of the two categories of plant material. Immediately after collection, the shoot tips were sprayed with 70% ethanol, then wrapped in ultra-pure water moistened tissue paper before dissection under a binocular microscope with a cold light source. From that stage onwards, all the manipulations were performed under a laminar flow hood, in aseptic

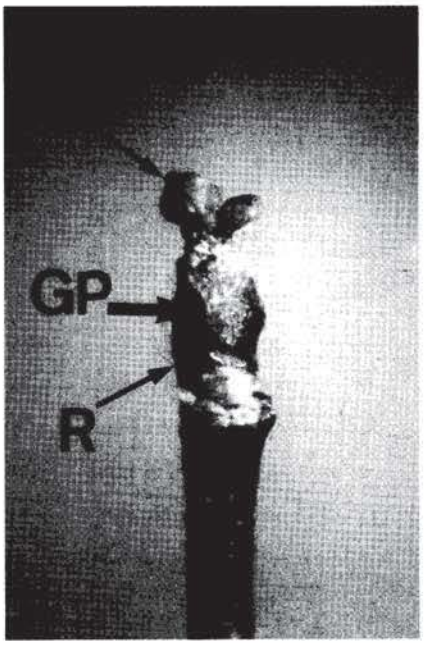

Figure 1. – *In vitro* micrografted shoot apex (S) of mature *Acacia mangium* ortet starting to elongate, with its "V" shaped basal wedge connected to the rootstock (R) tissues at the grafting point (GP).

conditions. After removal of the outer young phyllodes, the shoot apex, ranging from 300 µm to 400 µm in height, plus a short "V" shaped basal wedge of underlying tissues, was excised to be quickly and carefully inserted into a small vertical slit made in the center of the top cut surface resulting from the decapitation of the rootstock epicotyl, like for a miniaturized "cleft-graft" (HARTMANN et al., 1990), as illustrated in *figure 1*. The possibility to draw the rootstock out of the tube without damaging its root system was achieved thanks to the "Sorbarod" plug which facilitated the manipulations. Once grafted, the seedlings were returned to the initial environmental conditions taking care to remove the numerous axillary shoots produced by the stock to avoid competition with scion development.

Evaluation of grafting and micrografting success

Grafting success rate was established 2 months after grafting by recording the number of scions still alive out of the 15 grafts carried out per sampling date for the 2 categories of plant material.

Success rate for micrografting was defined as the number of grafts successfully established (scions still alive and eventually exhibiting some potential for further elongation) out of the 12 micrografts performed for each sample, 3 months after the date of micrografting. In addition, elongating scions were recorded and measured.

The data were statistically analyzed using the χ^2-PEARSON's test or the analysis of variance test (F-test) after the success rates had been transformed to arcsin $\sqrt{x}$ (SNEDECOR and COCHRAN, 1957), to determine significant differences ($P < 0.05$ level of probability) among the 2 origins.

Results

Data provided in *table 1* establish that *in vivo* grafting of *Acacia mangium* was greatly influenced by the age of the ortet ($P < 0.001$, F-test). On average 48.6% of scions coming from juvenile plant material were successfully grafted and developed into new shoots, whereas when using scions from mature ortets all the grafts failed. The marked difference between juvenile and mature plant material as regards their capacity for *in vivo* grafting, disappeared when using the shoot apex micrografting technique. It gave similar average success scores of 51.8% and 45.6% respectively. However, in contrast to *in vivo* grafting several of the successfully established *in vitro* micrografts were still resting 3 months after micrografting. The proportion was significantly higher for micrografts derived from mature ortets ($P < 0.001$, χ^2-test). Only 15 out of 36 had begun to resume growth at that time (*Figure 1*), as compared to 36 out of 42 for the juvenile plant material. Although the growing scions from the juvenile source were generally observed to elongate faster than their homologs from the mature ortets (*Figure 2*), the within sample variability did not allow to point out any significant difference in terms of scion length between the 2 age classes 3 months after micrografting.

Table 1. – In vivo grafting and *in vitro* micrografting success rates for scions collected at different dates from juvenile and mature *Acacia mangium* ortets and submitted exactly to the same experimental conditions.

IN VIVO GRAFTING			IN VITRO MICROGRAFTING		
Dates of scion collection	Success rate		Dates of scion collection	Success rate	
	Juvenile	Mature		Juvenile	Mature
4-12-1991	6/15	0/15	22-9-1994	2/12*	6/12
13-12-1991	5/15	0/15	5-10-1994	5/10	4/11
27-12-1991	2/15	0/15	2-11-1994	6/12	3/10
28-1-1992	12/15	0/15	24-11-1994	7/12	7/12
18-2-1992	8/15	0/15	16-12-1994	7/12	7/11
6-3-1992	11/15	0/15	21-12-1994	5/11	4/11
30-12-1993	7/15	0/15	29-12-1994	10/12	5/12
Average rate of success	51/105	0/105		42/81	36/79
% ± S.D.	48.6±4.9	0		51.8±5.5	45.6±5.6

*) initially 12 micrografts were performed for both ages of plant materials; some were lost due to fungal contaminations.

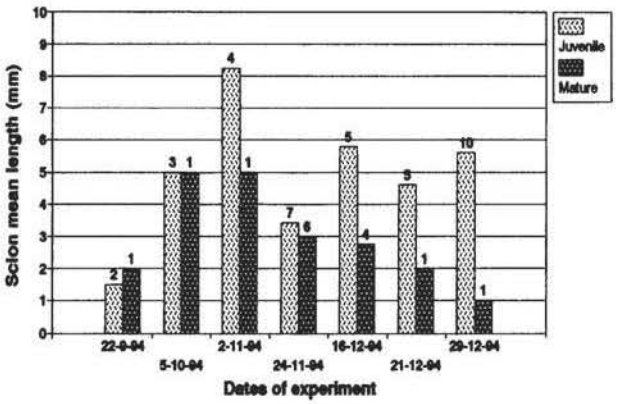

Figure 2. – Comparative mean length 3 months after micrografting of *in vitro* elongating scions collected from juvenile and mature *Acacia mangium* ortets at different dates and submitted exactly to the same experimental conditions. The number of scions which were elongating and measured is given for each sample.

Discussion

The current study indicates that although *Acacia mangium* can be grafted, success rates in *in vivo* conditions are rather low compared to other species and highly dependent upon the age of the donor tree. As a matter of fact, scion capacity to produce a callus, which can be considered as a characteristic wound response in plants (MOORE, 1981) and commonly reported as a prerequisite of grafting success (BARNETT and WEATHERHEAD. 1988; HARTMANN et al., 1990), was observed to be quite weak in this species, especially for mature plant material as was already noticed when studying rooting of cuttings (POUPARD et al., 1994; MONTEUUIS et al., 1995). The fact that flushing shoots produced by regularly hedged 3-year-old stock plants give rise to similar grafting scores as shoots from young seedlings (unpublished data) contrasts with scions taken from crown branches which tends to demonstrate the negative influence of increasing ontogenetical ageing (FORTANIER and JONKERS, 1976) of scions on their ability to be successfully grafted, in the same way as it has been observed for adventitious rooting potential of cuttings (MONTEUUIS et al., 1995).

The micrografting approach seems to neutralize the impact of ontogenetical ageing considered for the whole tree, since the 2 compared origins of micrografted shoot apices did not differ significantly in terms of potential for callus formation, giving rise to similar overall grafting success rates, although the apices originated from mature ortet crown were ontogenetically

older than the ones coming from juvenile seedlings. Particularly, in contrast with the 5 cm to 7 cm long scions used for *in vivo* grafting which all failed, 46% of the mature derived shoot apices produced a callus that allowed them at least to survive. This illustrates the possibility of finding within ontogenetically mature shoots that had already entered the flowering stage some less differentiated shoot tip tissues close to the apical meristem that could express a similar potential, at least for callus formation, as those produced by juvenile seedlings. This has been observed for other species also (MONTEUUIS, 1991). As far as the potential for grafting is concerned LACHAUD (1973) distinguished between ageing of scion tissues, referred to as "âge local" and ageing of the whole donor tree – "âge général". However, the fact that the successfully established micrografted apices of mature origin were more recalcitrant to elongate than their homologs originating from juvenile seedlings might be due to physiological and biochemical differences between the two origins of shoot apices, most probably caused by physiological ageing (LACHAUD, 1975; HACKETT, 1983; MONTEUUIS, 1989). With reference to other works (MONTEUUIS, 1987 and 1991), the possibility to reduce the size of the micrografted shoot apices to only the true apical meristem may help to counteract such negative effects of physiological ageing on the potential for organogenesis of the mature plant material. Experiments currently under way, although technically handicapped by the very tiny size of the *Acacia mangium* shoot apical meristem, may answer to what extent this will prove possible.

Conclusion

Shoot apex micrografting has to be considered as an helpful alternative to *in vivo* grafting for *Acacia mangium,* especially for plant material collected from the crown of mature ortets.

In addition to rejuvenation prospects, the importance of which has to be emphasized for clonally propagating true-to-type mature *Acacia mangium* genotypes, micrografting was found to be an efficient technique overcoming tissue culture contamination problems, particularly those of endogenous origin, which are to date serious impediments to micropropagation programmes of this economically important forest tree species.

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